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Department of Entomology, Kerala Agricultural University
Vellayani P.O, Thiruvananthapuram 695522, Kerala, India
Email : aae@kau.in; web: www.entomon.in

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Short Communication

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Descriptions of two new species of *Dipara* Walker from India with a revised key to the Indian Species (Hymenoptera: Chalcidoidea: Pteromalidae)

P.M. Sureshan*, P. Girish Kumar and S. Manikavasagam#

Zoological Survey of India, Western Ghat Regional Centre, Kozhikode 673006, Kerala, India;
 # Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram 608002 Tamil Nadu, India. Email: pmsuresh43@gmail.com

ABSTRACT: Two new species of *Dipara* Walker (Hymenoptera: Pteromalidae) are described based on the specimens from the Indian states of Tamil Nadu, Kerala and Nagaland. The key to the Indian species of *Dipara* is revised. © 2018 Association for Advancement of Entomology

KEY WORDS: Hymenoptera, Pteromalidae, *Dipara*, new species

INTRODUCTION

The genus *Dipara* Walker belongs to the subfamily Diparinae of Pteromalidae (Hymenoptera: Chalcidoidea) which is currently known by 54 species described worldwide, with 23 species known from the Oriental Region (Sureshan et al., 2014; Sureshan & Farsana, 2015; Sureshan et al, 2017; Noyes, 2017). Studies on further specimens of *Dipara* from the Indian states of Tamil Nadu, Kerala and Nagaland yielded two undescribed species which are described here. The key to the Indian species of *Dipara* published by Sureshan et al., 2017 is modified to accommodate the new species described here.

MATERIAL AND METHODS

The specimens were collected with sweep net and examined under Leica M 205C stereozoom microscope and images captured with the camera model Leica MC170 HD. Terminology used in the paper generally follows Gibson (1997) and the type specimens are deposited in the National Zoological Collections of Zoological Survey of India, Western Ghat Regional Centre, Calicut (ZSIK).

The following abbreviations are used in the text: fu_1–fu_7 = funicle segments 1 to 7; mv = marginal vein; OOL = ocellocular distance; pmv = postmarginal vein; POL = post-ocellar distance; smv = submarginal vein; stv = stigmal vein; Gt_1–Gt_6 = gastral tergites 1–6.

RESULTS AND DISCUSSION

Key to the Indian species of *Dipara* Walker (females)

1. Wings reduced (species brachypterous). ……..…… …… **2**

* Author for correspondence

– Wings fully developed (species macropterous). …….…..…….. **11**

2. Median area of propodeum with uniform longitudinal rugae, arranged in a sub-circular form; frenum represented by a very narrow area with small rugae (Sureshan et al., 2014: figs 10, 13); mesoscutum almost completely black with a characteristic 'W' shaped yellowish brown area. ……..... ..... ***D. yercaudensis*** **Sureshan**

– Propodeum without uniform longitudinal rugae, partly, irregularly carinated or with irregular areolae; scutellum always with frenum broader than above; mesoscutum not coloured as above, sometimes body completely brownish black. …….....…..… **3**

3. Mesoscutum with notauli not meeting in the posterior end. …..……...……. **4**

– Mesoscutum with notauli meeting in the posterior end.……..……..……. **9**

4. Mesoscutum blackish brown in distal two-thirds; carina of pronotal collar characteristically angulate and slightly broken in the middle (Sureshan *et al.,* 2014: fig. 14); forewing stump long and narrow reaching beyond tip of petiole. …..... ..... ***D. angulata*** **Sureshan & Nikhil**

– Mesoscutum without blackish brown colour as above, sometimes body uniformly brownish black, then forewing stump long and broad, reaching beyond tip of petiole otherwise; forewing stump very short and not reaching petiole. ……..….....…..... **5**

5. Forewing stump 4.3× as long as broad, reaching beyond tip of petiole; petiole stout, finely reticulate, 1.6× as long as broad; propodeum with characteristic median carina, plicae and costulae (Sureshan *et al.,* 2014: fig. 19)…….....…… ***D. venkati*** **Sureshan**

– Forewing stump very short and narrow not reaching or just touching base of petiole; petiole slender, longitudinally carinate, more than 1.6× as long as broad; propodeum with or without median carina, plicae and costulae and not as above. ….....…..……..…….. **6**

6. Propodeum with baso-medial area between plicae conically elevated up to middle, surface not shiny, with longitudinal and transverse carinae and striae; wing stump extending well beyond the hind margin of scutellum, touching base of petiole and with 5 or 6 bristle …… **8**

– Propodeum (Sureshan *et al*., 2014: Fig. 18) with baso-medial area between plicae not conically elevated up to middle, surface almost shiny, sometimes with very weak striations; wing stump very short, not reaching much beyond hind margin of scutellum or sometimes hardly reaching hind margin of nucha, then with only 3 bristles. ……..….....…. **7**

7. Forewing stump very short, only a little longer than tegula, not extending much beyond hind margin of scutellum; forewing with 2 bristles; OOL almost as long as POL; antenna with scape as long as eye, pedicel slightly longer than fu_1; general body colour yellowish brown. ..... ***D. intermedia*** **Sureshan & Narendran**

– Forewing stump 5.2× as long as tegula, hardly reaching tip of nucha, forewing with 3 bristles; OOL 1.2× POL; OOL almost as long as POL; antenna with scape 0.8x as long as eye, pedicel distinctly longer than fu_1 (Sureshan et al. 2017: Figs 1(B),1(D), 1(E) ; general body colour dark honey brown.....***D. tamila*** **Sureshan *et al.***

8. Body length, 2.6 mm; upper face and vertex distinctly reticulate; POL equal to OOL; antenna with fu_6 and fu_7 whitish yellow as clava; fl_2– fl_5 dark brown; wing stump with 5 bristles; hind coxae reticulate……. ..... ***D. eukeralensis*** **Özdikmen**

– Body length, 1.5 mm; upper face and vertex almost shiny, only weakly reticulate; POL slightly shorter than OOL; only fl_7 whitish yellow as clava; fu_3– fu_6 brown; wing stump with 6 bristles; hind coxa striate reticulate. .....***D. mohanae*** **Narendran & Sureshan**

9. Mesoscutum with distinct black patch covering almost posterior two-thirds; pronotum with a distinct transverse carina; forewing stump with one short and one long bristle..... ..... ***D. thirumalaii*** **Sureshan**

– Mesoscutum with black patch on posterior half of scapulae only; pronotum with or without transverse carina; forewing stump with 3 or 4 setae..... ..... **10**

10. Propodeum without median carina; antenna with fu_6 partly whitish yellow as clava; forewing stump with 4 setae; gaster swollen with Gt_3–Gt_6 not very short, and yellowish brown with distinct yellow band overlapping Gt_1 and Gt_2; Size 1.8–1.9 mm .... ***D. gastra*** **(Sureshan & Narendran)**

– Propodeum with weak median carina in posterior third, antenna with fu_6 completely brown; forewing stump with 3 setae; gaster narrow and compressed with Gt_3– Gt_6 short, and almost brown with epipygium and ventral part paler; Size 1.45–1.6 mm .... ***D. malabarensis*** **(Narendran & Mini)**

11. Pronotal collar with separate long and stout bristles near posterior margin in addition to pubescence. **15**

– Pronotal collar without separate long and stout bristles near posterior margin in addition to pubescence. **12**

12. Mesoscutum posteriorly with a black or bluish black patch in lower half of scapulae; petiole 2–2.6× as long as broad in dorsal view **13**

– Mesoscutum posteriorly without black or bluish black patch in lower half of scapulae, sometimes black patch covering all the three lobes in posterior third; petiole 1.63× as long as broad in dorsal view **14**

13. Scapulae with the bluish black patch distinct and covering almost half length in the lower half; notauli closely converging posteriorly; petiole 2× as long as broad in dorsal view; pronotal collar carinated anteriorly..... .....*D. debanensis* **Sureshan**

– Scapulae with the black patch small, and not sharp, and covering only posterior third; notauli not closely converging posteriorly as above; petiole 2.6× as long as broad in dorsal view (Sureshan et al., 2014: figs 2, 20); pronotal collar not carinated anteriorly. ..... ***.....D. andamanensis*** **Sureshan & Farsana**

14. Mesoscutum without any black patch or band, bristles a little above centre; reticulation of body fine; antenna with fu_4–fu_6 brown (fu_4 partly). ..... ….***D. miniae*** **Narendran & Sureshan**

– Mesoscutum with a broad black patch covering all the three lobes in posterior third, bristles in the centre; reticulation of body coarse; antenna with fu_4– fu_7 brown..... .... ***D. nigriscuta*** **Sureshan**

15. Scrobe long, separated from front ocellus by a distance as long as the diameter of front ocellus, exceeding well over middle length of eye from toruli. **16**

– Scrobe shorter, at the most reaching mid-level of eyes from toruli. **22**

16. Antennae inserted on an elevated point on face, head very narrow in profile view with eyes small (Fig. 1); notauli meeting posteriorly to form a broad 'V' touching transscutal articulation (Fig. 2). ..... ***D. elevata*** **Sureshan sp. nov.**

– Antennae inserted not on much elevated point as above, head more thicker in profile than as above, eyes not small as above; if notauli meeting posteriorly then scutellar frenum shiny…….... **17**

17. Scutellar frenum completely shiny except for the crenulate foveae on the posterior margin; notauli deep and merging posteriorly to form a broad "V", mid lobe of mesoscutum little above than the side lobes (Fig.6)..... ..... ***D. nitidofrena*** **Sureshan sp. nov.**

– Scutellar frenum not shiny, always with longitudinal ridges or sculpture; mesoscutum and notauli different…….... **18**

18. Petiole smooth and shiny without longitudinal carina, with maximum posterior width 1.13× dorsal length; frenal area of scutellum shorter than scutellar area in front; body large, length 4.5 mm. …… ***D. sringericus*** **(Narendran)**

– Petiole with distinct longitudinal carina or reticulation, with maximum posterior width 0.7–1.0× dorsal length; frenal area almost as long as scutellar area in front; body small, length 1.5–2.7 mm. ……..…… **19**

19. Antenna with anellus wide, distinct; fu_1 anelliform, without sensilla (Sureshan & Farsana, 2015: Fig. 5); gastral petiole as long as broad in dorsal view; general pubescence on head and mesosoma long in the form of thin bristles (Sureshan & Farsana, 2015: Fig. 1)... ***D. ponmudiensis*** **Sureshan & Farsana**

– Antenna with anellus not wide as above, less distinct; fu_1 not anelliform, with sensillae; gastral petiole distinctly longer than broad; general pubescence of the body short, not in the form of bristles as above……...….. **20**

20. Petiole long, 1.7× as long as broad in dorsal view, almost half length of hind coxa, dorsally mostly reticulate and with carinae only in hind part (Sureshan et al, 2014: Fig. 17); face without metallic blue reflection. (Size 2.7 mm) ……..…….. ***D. nigra*** **Sureshan**

– Petiole short, 1.2–1.4× as long as broad in dorsal view, without reticulation, only longitudinally carinate, carinae sometimes weak medially; face with distinct metallic blue reflection. …… **21**

21. Petiole short, 1.2× as long as broad in dorsal view, and with a pair of setae very close to anterior margin; pronotal collar with a row of four strong setae near posterior margin; bristles on the mid lobe of mesoscutum little below middle; fore wing almost hyaline..... ……..... ***D. hayati*** **Sureshan**

– Petiole long, 1.4× as long as broad in dorsal view, and with a pair of setae almost in the middle (Sureshan et al, 2014: Fig. 16); pronotal collar with a row of two strong setae near posterior margin; bristles on the mid lobe of mesoscutum little above middle; fore wing smoky..... ..... ***D. kannurensis*** **Sureshan & Farsana**

22. Forewing with three infumate patches; petiole a little longer than half length of hind coxa; axillae and pronotum pink; head mostly brownish pink with vertex and occiput darker. …… ***D. bouceki*** **(Narendran)**

– Forewing without inhumations, hyaline; petiole one-third as long as hind coxa; axillae, pronotum and head black. …….. ..... ***D. keralensis*** **(Narendran)**

DESCRIPTION OF SPECIES

1. Dipara elevata Sureshan sp. nov.

LSID urn:lsid:zoobank.org:act:3EE9C6D0-2092-4A22-BCE1-A2EAAEB9A7A3

(Figs. 1-4)

Holotype; *Female*: Length 3.2 mm (without ovipositor), ovipositor exerted 0.84 mm. (Paratype female Reg. No. 10253: Length 2.0 mm (without ovipositor), ovipositor exerted 0.2 mm; Paratype female Reg. No. 10254: Length 2.2 mm (without ovipositor), ovipositor exerted 0.5 mm). Body honey brown except upper half of propodeum and gaster dorsally and laterally (on upper half) blackish brown; eyes silvery white; ocelli reflecting white;

Figs 1-4 ***Dipara elevata*** Sureshan sp. nov. Figs 1-3 Holotype female. 1, Body in profile; 2, Head, mesosoma and petole in dorsal view; 3, Metasoma in dorsal view. Fig. 4 Paratype male in profile. Figs 5-8 ***Dipara nitidofrena*** Sureshan sp. nov. Holotype female. 5, Body in profile; 6, Mesosoma in dorsal view; 7, Propodeum and petiole in dorsal view; 8, Metasoma in dorsal view.

area of ocellar triangle black. Antennae testaceous except clava dark brown. Bristles of the body black. Legs uniformly testaceous. Wings almost uniformly smoky except basal cell and speculum hyaline.

Head (Figs 1, 2): In dorsal view 2.06× as broad as long and in frontal view 1.2× as broad as long; distinctly striate reticulate, clypeal and paraclypeal areas shiny, anterior margin of clypeus straight. Eyes small, length 1.5× width. Malar grooves distinct, carinated in the upper 2/3 portion; scrobe deep, shiny, separated from front ocellus by one ocellar diameter. Vertex straight, narrow with three pairs of strong bristles; occiput acutely declivous with occipital carina far below; POL 1.2× OOL. Antennae slender, inserted on a raised point, little below lower margin of eyes; interantennal area conically elevated with a median carina reaching little above lower margin of eye; toruli separated by a distance 1.5× the individual diameter; scape slender, reaching front ocellus, length 1.4× eye length, pedicellus plus flagellum1.4× width of eye; pedicel length 3.2× width; clava 2× as long as broad and as long as 2.5× preceding segments combined. Relative lengths: fu_1 9.5, fu_2 8, fu_3 7.5, fu_4 7, fu_5 7, fu_6 7, $fu_7$7, clava 19.

Mesosoma (Figs.1, 2): Length 1.5× width. Pronotum with a separate row of 10 strong bristles, collar transversely reticulate with a smooth band posteriorly, shiny on lower lateral aspect. Prepectus broad, shiny, longer than tegula. Mesoscutum

distinctly and transversely reticulate, width 1.7× median length, notauli distinct, posteriorly meeting to form a broad 'V' touching transscutal articulation; bristles on the midlobe distinctly below middle. Scutellum length 1.1× width, frenal area with distinct rugae, area in front distinctly reticulate, first pair of scutellar bristles in the middle of reticulate area; frenal area almost as long as area in front. Propodeum width 2.4× median length with an anterior triangular elevated area in the form of a tubercle which continued as a median carina; plicae distinct only posteriorly which is connected with a strong transverse carina, remaining area of propodeum uniformly with irregular rugae; spiracles small, oval, separated far away from the posterior margin of metanotum, callus almost shiny with sparse long setae. Mesopleuron shiny except anterior margin with transverse rugae. Metapleuron shiny. Hind coxae with transverse rugae in the basal half and reticulate in the distal half; femur finely reticulate; tibia distinctly reticulate. Relative length of hind coxa 37, femur 49, tibia 59, tarsus 39. Forewing 3.6× as long as broad, marginal fringe long, pubescence distinct, speculum open below, extended below mv almost up to base of stv. Relative lengths of smv 46, mv 79, pmv27, stv 12.

Metasoma (Fig. 3): Gaster (including petiole) 1.4× as long as head plus mesosoma combined. Petiole with distinct longitudinal carina on posterior half and reticulate on other areas, a pair of backwardly directed hairs near base, length 1.4× width. Gaster 2.8 × as long as broad in dorsal view (without petiole); Gt_1 0.4× as long as whole length of gaster, Gt_2, Gt_4 , Gt_5 and Gt_6 almost equal; Gt_3 less than half of Gt_4; ovipositor sheath and ovipositor distinctly exerted out. Hypopygium reaching hind margin of Gt_1.

Male (Fig. 4): Length 1.7 mm. Morphologically entirely different from female in having body colour almost black except gaster brownish black, body slender, antennae with 9 pedunculate funicular segments and clava with long bristles, apart from scape, pedicel, and transverse anellus; head almost completely shiny; mesoscutum with notauli meeting posteriorly to form a broad "Ú" rather than "V"; scutellar frenum with longitudinal rugae on sides; propodeum with anterior elevated area not prominent; gastral petiole very long and uniformly reticulate; gaster short and compressed.

Material examined: Holotype: Female, INDIA: Tamil Nadu, Yercaud, 06.iii.2014, Coll. S. Manickavasagam, Reg. No. ZSI/WGRC/IR/INV/5080; Paratype: 1 male, data same as that of holotype, Reg. No. ZSI/WGRC/IR/INV/5081; 1 Female, Kerala, Idukki district, Periyar Tiger Reserve, Manalar, 7.iv.2013, Coll. P.M. Sureshan, Reg. No. ZSI/WGRC/IR/INV/10253; 1 Female, Kerala, Idukki district, Idlimotta, 25.v.2014, Coll. P.M. Sureshan, Reg. No. ZSI/WGRC/IR/INV/10254.

Etymology: The name of the species is derived from the latin word elevatio = raised and in having antenna inserted on an elevated point on face.

Remarks: This species is unique among other Oriental species in having antennae inserted on an elevated point on face which is not much prominent in other species, head in profile view very narrow; notauli meeting posteriorly to from a broad 'V' and, narrow forewings and male with pedunculate funicular segments. It runs into the couplet 14 of the key to Oriental species of *Dipara* by Sureshan & Farsana (2015) and resembles *D. nigra* Sureshan in general morphology but differs from it in having slender antennae, different body colour, wings and gaster.

2. *Dipara nitidofrena* Sureshan sp. nov.

LSID urn:lsid:zoobank.org:act:CF8FCE5C-9A22-4017-872F-9B8A987DE1FC

(Figs. 5 - 8)

Holotype: *Female*: Length 2.00 mm. Body pale yellow except for the following: Head blackish brown, antennae pale testaceous except clava brown, eyes silvery, mesoscutum and scutellum blackish brown, gaster except Gt_3 dark brown dorsally and laterally on upper half of Gt_1, wings hyaline, pubescence of body pale brown and pubescence of wing brown.

Head (Fig.5): In dorsal view 2.1× as broad as long and in frontal view 1.2× as wide as long, finely engraved reticulate, almost shiny. Clypeus smooth, anterior margin straight. Malar grooves finely indicated. Vertex almost straight, POL subequal to OOL (7:8); occipital area finely reticulate with a fine carina far below. Antennae inserted slightly below lower margin of eyes, below middle of face, scape length 1.2× eye length, eye length 1.3× width; pedicellus plus flagellum length 1.4× width; pedicel long 2x as long as fu_1; fu_1-fu_4 almost quadrate; fu_5-fu_7 transverse; clava 2× as long as broad, slightly shorter than three preceding segments combined, all funicular segments with one row of long sensillae.

Mesosoma (Figs. 5, 6, 7): 1.6× as long as broad. Pronotum almost completely shiny, collar distinctly carinated anteriorly with a separate row of strong bristles. Mesoscutum distinctly reticulate, reticulation finer anteriorly; notauli deep towards posterior end and almost merging together to form a broad "V" so that the mid lobe little raised than the lateral lobes; bristles on the mid lobe located below middle; mesoscutum width 1.4× length. Scutellum similarly sculptured as on mesoscutum, frenal area shiny except for small rugulate foveolae on the posterior rim,1.5× as broad as median length, frenal area half as long as area infront. Prepectus shiny, longer than tegula. Mesopleuron and metapleuron shiny except for a row of rugulate foveolae in the anterior margin of the former. Propodeum 1.9× as broad as median length, there is triangular elevated area anteriorly in the form of a blunt spine which continued as median carina, spiracles small, round, separated far away from posterior margin of metanotum, plicae present only posteriorly which is terminated as a lateral blunt spine after joining with a short transverse carina, remaining median area with regular longitudinal carinae; callus smooth with scattered hairs. Legs slender, hind coxae with transverse rugae, fore and mid coxae almost shiny. Relative lengths of hind coxa 28, femur 38, tibia 41, tarsus 13. Forewing 3.8× as long as broad, narrow, basal one third portion almost bare except for few setae on basal hairline; marginal fringe long. Relative lengths of veins: smv 32, mv 48, pmv 19, stv 6.

Metasoma (Figs. 5, 8): Gaster 1.1× as long as head plus mesosoma combined, length 2.7× width in dorsal view; petiole uniformly longitudinally carinate and reticulate with a pair of long white hairs in the upper 1/3 portion, length 1.4× width; a distinct yellow band covering Gt_3 & Gt_4; hypopygium not reaching tip of Gt_1.

Material examined: Holotype, female, INDIA: Nagaland, 19.i.2015, Coll. S. Manickavasagam; Reg. No. ZSI/WGRC/IR/INV/5082.

Etymology: The species name is derived from latin word nitidus = shiny and in having a shiny scutellar frenum.

Remarks: This species is unique among other Oriental species in having a completely shiny scutellum which is not found in other species, and mesoscutum with deep notauli merging posteriorly to form a broad 'V' and lateral lobes situated little below median lobe. It runs into couplet 14 of the key to Oriental species of *Dipara* by Sureshan and Farsana (2015) and resembles *D. andamanensis* Sureshan which is having partly smooth frenum, but differs from in having different gaster, antennae, body colour and propodeum.

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Quantitative and qualitative changes in proteins and shift in the utilization of amino acids for cuticle sclerotisation and energy release during development in *Culex quinquefasciatus*

R.V. Ayana Gayathri and D.A. Evans*

Department of Zoology, University College, Thiruvananthapuram, India.
Email: drevansda@gmail.com

ABSTRACT: Whole body protein of *Culex quinquefasciatus* showed a gradual increase during development and the content in 1st instar larvae was 3% and in adults it was 5% on fresh weight basis. The amount of total free amino acids showed a gradual increase up to 4th instar larvae but a sharp decline in pupae and adults, because of the mobilization of amino acids for the formation of new proteins in pupae. Appearance of new protein bands was not prominent during larval development but pupation resulted in origin of new protein bands. Protein profile of adult male and female did not exhibit marked difference in SDS-PAGE. Activity of alanine aminotransferase showed a gradual elevation during larval development but an antiparallel pattern was shown by another related enzyme aspartate aminotransferase. The ratio of activity of AlAT: AsAT, which is an index of utilization of amino acids in Krebs cycle via keto acids, was always below 0.3 in larvae, elevated to 0.6 in adults, which may be a part of flight adaptation. Maintenance of high activity of glutamate dehydrogenase (GDH) by adult mosquitoes in comparison with their larvae is also a part of flight adaptation because GDH- Transaminase system is responsible for supplying pyruvic acid and oxaloacetic acid from amino acid pool to Krebs cycle. Activity of phenoloxidase (tyrosinase), which is an index of melanization of cuticle and defense against parasites, showed a sharp increase from first instar to adults. As the melanization and sclerotisation of *C. quinquefasciatus* were practically completed by the 48 hour of eclosion, the activity of phenol oxidase showed a gradual retardation.

KEY WORDS: *Culex quinquefasciatus*, alanine aminotransferase, glutamate dehydrogenase, phenoloxidase, melanization.

INTRODUCTION

The holometabolous group of insects has distinct larval and pupal stages and undergoes some of the most complex transformations seen in animal kingdom(Sehnal *et al*., 1996, Truman and Riddiford, 1999).The major morphogenetic events, such as determination of organ systems during embryogenesis, growth and moulting during larval life,as well as transformation from larva to pupa at the time of metamorphosis, are accompanied by characteristic variations in the patterns of amino acids, peptides and proteins (Chen, 1966). Mosquitoes are very peculiar in having highly mobile pupae with very short pupal life of 48-60 hours within which the aquatic detritivore is converted into a sanguivorous disease vector.Information on the metabolism of amino acids in insects like

* Author for correspondence

Periplaneta (Sacktor, 1978), *Schistocerca* and *Drosophila* (Schneider and Chen, 1981) is known, but that of mosquito and its developing stages is not clearly understood so far. Studies in other insects such as *Oecophylla smaragdina* (Vidhu and Evans, 2014) and *Oryctes rhinoceros* (Nayar, 2015) showed that glucose, the most important energy releaser of vertebrates, the content of which remained unaltered in extra energy necessitating stressed states such as continuous biting on the intruder till death (Vidhu and Evans, 2015) and exposure to hypothermia (Nayar, 2015). Contrary to it, the enzymes related to conversion of amino acids to keto acids and mobilization of keto acids into Krebs cycle became greatly elevated in stressed states. Also, the activity of phenoloxidase enzyme has been elucidated during the various stages of life cycle. Physiological and biochemical studies on insect development can provide a better understanding of the mechanisms of hormone action, protein synthesis, growth and differentiation. So it was proposed to study the protein profile and biochemical changes in amino acid metabolism during course of development using *Culex quinquefasciatus* as a model system.

MATERIALS AND METHODS

Collection of *Culex* egg rafts: Egg rafts of *Culex* mosquito were collected by keeping 3 litres of water containing 2 eggs of domestic fowl in plastic buckets (5L), placed at damp corners of the College Campus. Mosquitoes started laying eggs after 2-3 days, once foul smell emanated from them and egg rafts were collected.

Rearing of *Culex* larvae, pupae and adults: Collected egg rafts were transferred to plastic pans (20 cm diameter and 8.5 cm depth), containing 500 ml of the same medium in which the eggs were laid, kept at insectarium in the department. Water was changed once every 3 days. Hatched out different instars and pupae were collected using droppers. Emerging pupae were transferred to mosquito cages for their development into adults. Adults were provided with 10% sucrose solution ad libitum.

Experimental organism: *Culex quinquefasciatus* was identified by the methods of Barraud (1934) and Harbach (2014). Adults and developing stages of *C. quinquefasciatus* reared in the insectarium of the department was used for the biochemical studies. The temperature of the insectariums ranged between 26-30°C. The different larval instars were separated as described by Tripathy and Dash (1988).Mosquito larvae and pupae were separated from the rearing colony and washed four times using dechlorinated tap water and finally by distilled water. With respect to developing stages, the whole body was processed for the assays. In the case of adults, appendages, wings, legs, antennae and mouth parts were removed before homogenization by keeping them in ice cold condition. Three day old mosquitoes fed with 10% Sucrose were used for the studies.

Biochemical analysis and SDS- PAGE:100mg body weight of tissue was weighed out for all the stages of development. They were blotted using filter paper and transferred into a glass homogenizer containing appropriate volumes of ice cold buffer. For each enzyme assay, the buffer mentioned in the standard procedure was used for extracting the enzyme from the tissue. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4ºC (Eppendorf) and supernatant was taken. The pH and molarity of the buffer used was in accordance with standard procedure. Total protein was estimated by the method of Lowry *et al*. (1951) using BSA as standard. Estimation of total free amino acids was done according to Spies (1957). SDS- PAGE electrophoresis of whole body homogenate was carried out by the method devised by Laemmli (1970). It was carried out using 12% Polyacrylamide gel, pH 8.8. Acrylamide: Bisacrylamide ratio was 30:1. All protein samples contained 100µg of protein and were pretreated with 10% SDS and 1% mercaptoethanol at 95ºC for 3-5 minutes. The gel was run at 120V until the tracking dye (Bromophenol blue) was leaving the gel. Gel was stained in Coomassie Brilliant blue R250 overnight and destained in 7% acetic acid and photographed using Transilluminator (Biotech, Yercaud, India).

Assay of Aspartate aminotransferase (AsAT, EC. 2.6.1.1) and Alanine aminotransferase (AlAT, EC. 2.6.1.2) was done according to the method of Reitman and Frankel (1957). Glutamate dehydrogenase (GDH, EC. 1.4.1.4) and phenoloxidase (EC. 1.14.18.1) were assayed according to methods of Strecker (1955) and Lerch (1987) respectively. All chemicals were purchased from Sigma Aldrich Co.

Statistical analysis: Data obtained were subjected to statistical analysis using SPSS 22.0 software for Windows. One way analysis of Variance (ANOVA) was done followed by Duncan's Multiple Range test (DMRT). Data was considered to be statistically significant, if $P \leq 0.05$.

RESULTS

Total protein and total free amino acids:

Total protein during course of development ranged between 3% and 5% of fresh body weight,showing a gradual increase from 1st instar to adult mosquitoes. Adult females had the maximum content of total protein among the different stages of life cycle.The amount of total free amino acids on the other hand showed a sharp increase up to 4th instar larvae. There was a decline from pupa to adultsand the results are also shown in Fig. 1.

SDS - PAGE Analysis:

Elevation of protein content from first instar to adults was substantiated through a significant change in the protein profile in 1D gel electropherogram (Fig. 2). The protein profile of pupa was conspicuously different from the rest with a protein band within 66-97.4 kDa molecular weight of large intensity and volume which was absent in different larval instars and adults. The protein profiles of males and females resolved into 21 bands and bands were of the same molecular weight range.

Transaminases:

The activity of alanine aminotransferase (AlAT)showed a significant elevation from 1st instar to 4th instar, declined in pupa and reached maximum in males and females. Aspartate aminotransferase (AsAT) activity showed antagonistic response from first instar to pupae and an elevation in males and females. The AlAT/AsAT ratio upto pupae was always ≤ 0.3, but the ratio in adult males and females was 0.6. Maximum ratio

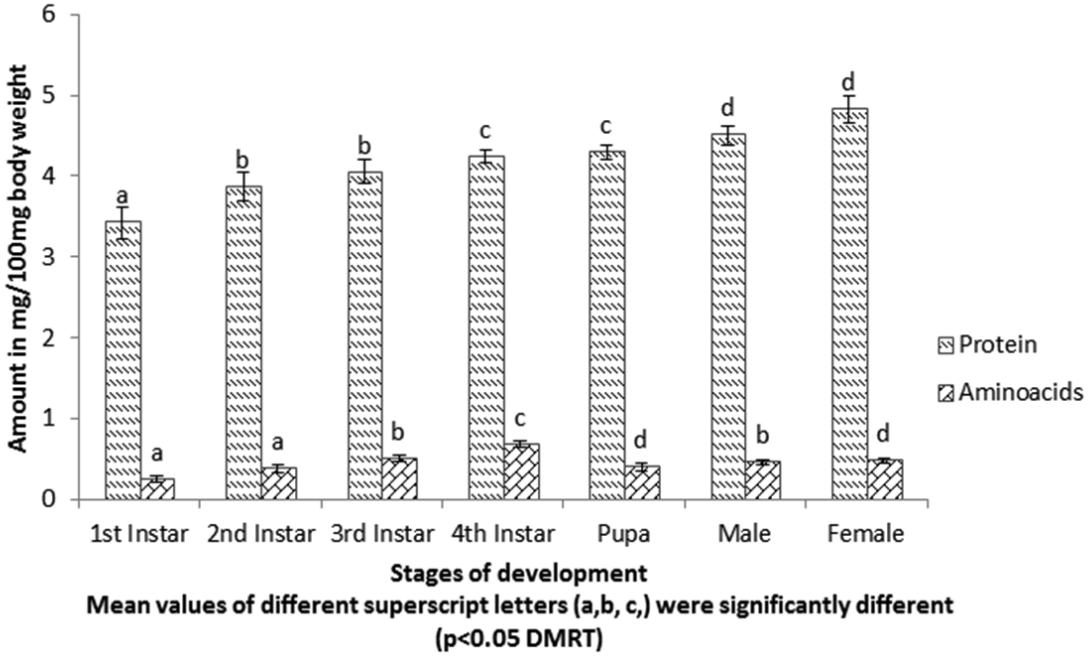
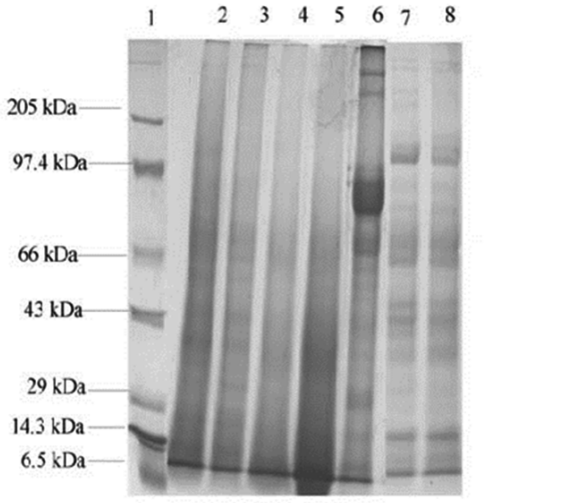


Fig.1 Total protein content and total free amino acids in *Culex quinquefasciatus* during course of development

of transaminase activity was shown by adult females i.e 0.65 (Table 1).

Glutamate dehydrogenase:

Activity of glutamate dehydrogenase (GDH) also showed a sharp decrease from 1st larval instar to pupal stage, where it had the minimum activity and showed an exponential elevation in adult males and females (Fig. 3). Maximum activity of glutamate dehydrogenase was shown by adult females.



. Lane 1- Marker, Lane 2- 1st instar larvae, Lane 3- 2nd instar larvae , Lane 4 – 3rd instar larvae, Lane 5- 4th instar larvae, Lane 6 – Pupae, Lane 7- Adult Male and Lane 8- Adult female.

Fig. 2. ID SDS - PAGE Electropherogram of *Culex quinquefasciatus* during course of development

Table 1. Activity of alanine aminotransferase and aspartate aminotransferase during the course of development of *Culex quinquefasciatus.*

Stage of development	Activity of Transaminases		AlAT/AsAT Ratio
	AlAT/GPT*	AsAT/GOT**	
1st Instar	0.2903 ± 0.0585^{a}	3.8517 ± 0.1354^{a}	0.08
2nd Instar	0.518 ± 0.1427^{b}	3.6953 ± 0.1276^{b}	0.14
3rd Instar	0.754 ± 0.1424^{c}	3.2651 ± 0.1287^{c}	0.23
4th Instar	1.0512 ± 0.1286^{d}	3.0922 ± 0.1667^{c}	0.33
Pupa	0.7282 ± 0.0698^{a}	$2..8333 \pm 0.0973^{d}$	0.25
Male	1.8052 ± 0.1483^{c}	2.9027 ± 0.2001^{c}	0.6
Female	2.011 ± 0.1449^{e}	3.1017 ± 0.1943^{c}	0.65

* Activity is expressed in micromoles of Pyruvate liberated/min/mg of protein.
** Activity is expressed in micromoles of Oxaloacetate liberated/min/mg of protein.
All values are Mean ± SE; n=6.
In Columns, Mean values of different superscript letters (a, b, c,) were significantly different (p<0.05 DMRT)

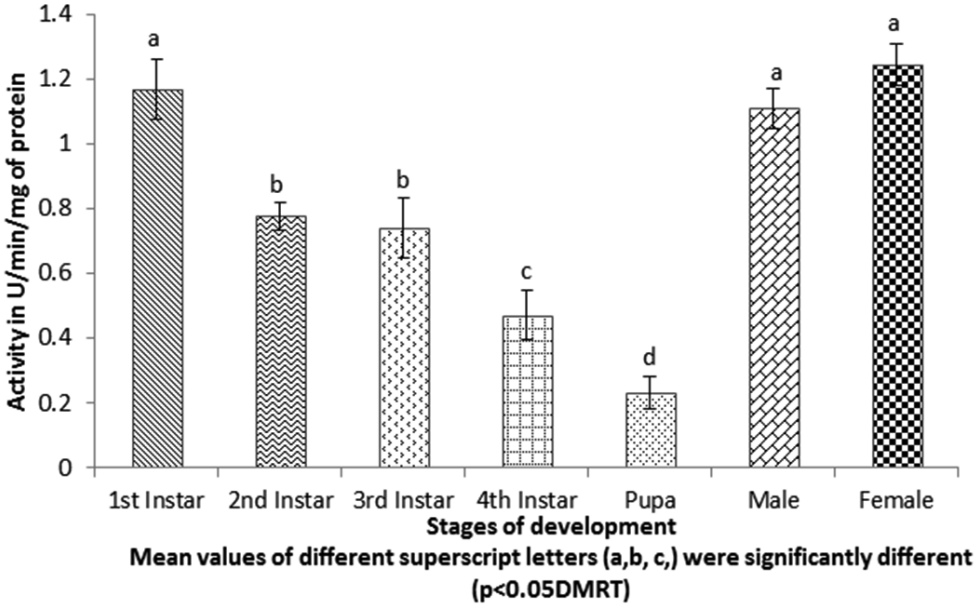


Fig. 3. Activity of glutamate dehydrogenase in *Culex quinquefasciatus* during course of development

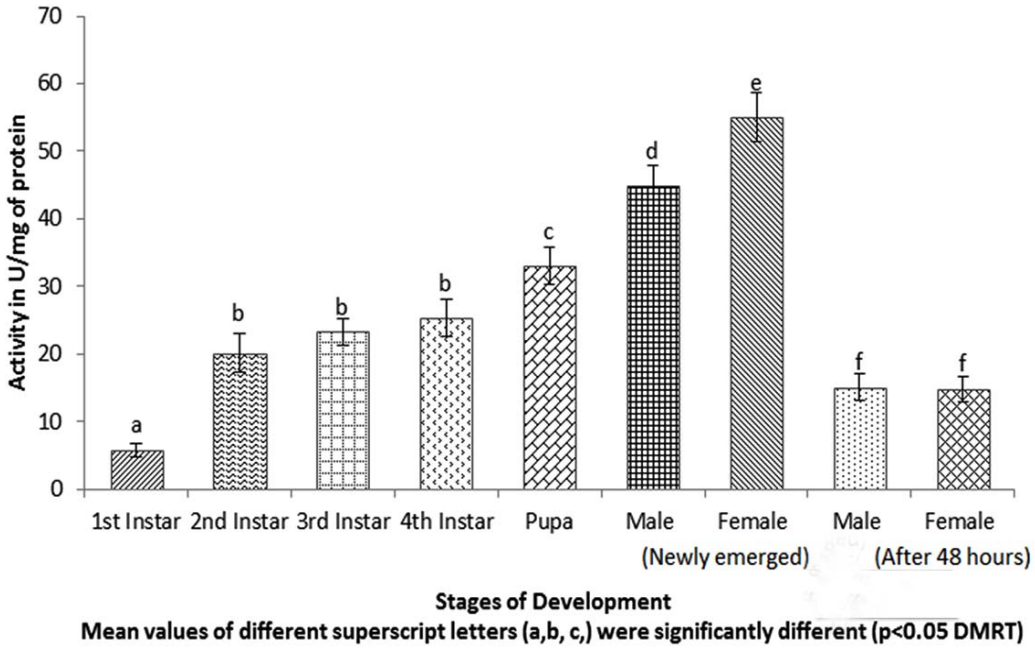


Fig. 4. Activity of phenoloxidase in *Culex quinquefasciatus* during development

Phenoloxidase:

Activity of phenoloxidase exhibited significant variation among the different developmental stages and activity showed a sharp elevation from 1st instar to adults, with adult females on emergence showing the maximum activity. However, the trend was reversed in both males and females after 48 hours on emergence where the activity declined sharply (Fig. 4).

DISCUSSION

Profound changes in protein turn over and amino acid metabolism take place at various periods during insect development (Chen, 1966). In the present study, qualitative and quantitative changes in the protein and catabolism of amino acids in relation to energy metabolism and activity of phenol oxidase during course of development in filarial vector, *C. quinquefasciatus* has been investigated.

In *C. quinquefasciatus*, there is a gradual increase in the total protein content from first instar larva to adult mosquitoes, with adult females showing the maximum amount of protein which was identical to the previous reports in *C. quinquefasciatus.* The high protein content of fourth instar larvae compared to other instars clearly agreed with the physiology of that stage. This instar has to store large quantities of proteins for pupal life. It is a well established fact that pupae are metabolically very active but they do not feed as they are not provided with functional mouth parts (Mozzelli, 1955). Increase in total protein in pupal stage appears to be directly associated with the conversion of insoluble larval cuticular and other proteins into soluble ones suggesting histolysis and also metabolism of glycogen and chitin. Elevated activity of Cathepsin D, an enzyme directly involved in the internal reorganization of proteins and organs have been reported in pupae of *Oryctes rhinoceros and Oecophylla smaragdina (*Nayar and Evans, 2011; Vidhu, 2014). The observations made by Pant and Kumar (1980) in dipteran flesh fly *Sarcophaga ruficornis* and *Trabala vishnou* (Gakhar *et al*.,1997) during metamorphosis of final larvae into pupa very well agreed with wide spread histolysis and internal reorganization during pupal life.Increase in total proteins in non-blood fed and newly emerged *C. quinquefasciatus* may be due to the process of histogenesis of adult tissue and

the observation very much agreed with the investigations in the malarial vector *Anopheles stephensi* (Gakhar *et al*., 1997).

Elevation in total protein content during course of development in *C. quinquefasciatus* was substantiated in 1D SDS-PAGE electropherogram. The intensity of protein bands of different stages gradually increased from first larval instar to fourth relating to increase in protein content obtained in quantitative estimation. The protein profile of pupae was found to be entirely different from earlier instars with the appearance of a high intensity band between 97.4 and 66kDa molecular weight range.Similar observations were reported by previous investigators in *Megachile rotundat*and suggested that these bands may be regulating pupation (Rank *et al*., 1982). Identical observations were obtained in the electropherogram of developing *A. stephensi* by Gakhar *et al.* (1997). Adult male and females had a distinct protein banding pattern with well- defined bands just like in pupa. The protein profile of male and female *C. quinquefasciatus* showed no distinct dissimilarity in number and intensity of protein bands.

In the present study, there is a slight, but gradual elevation in the amount of total free amino acids from first instar larvae to fourth.In the pupae, the total free amino acids decreased sharply and elevated further in adult males and females.The maximum amount of total free amino acids is shown by 4th instar larva. Similar results were obtained by Chen (1958) in various larval and pupal stages of *C. pipiens* and by Pant and Kumar (1980) in dipteran flesh fly*, Sarcophaga ruficornis*. Increase in free amino acids during larval development suggests the degradation of the ingested dietary proteins and their further utlilization for the formation of larval structures. At the onset of pupation, the significant depletion in free amino acids indicates their involvement in the synthesis of cuticular proteins for the formation of puparium. In adults, on the other hand, there occurs a gradual degradation of stored proteins into amino acids which eventually get involved in the formation of adult tissues. Besides, these amino acids have been reported to participate in energy metabolism in some insects. At metamorphosis, the level of total free amino acids stays either rather constant in some insects or exhibits an initial rise followed by a fall during later pupal development. In the latter case, the variation is interpreted as reflecting the break-down of larval tissues and formation of adult tissues. A part of the amino acids in the developing pupa can be oxidized and converted to fatty acids and carbohydrates in *Lucilia cuprina* (Crompton and Birt, 1967).

In the present investigation, the activity of alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT) differed during the course of development in *C. quinquefasciatus*. AlAT showed a significant elevation from 1st instar larvae to fourth, declined in pupa and rose again in adult males and females. Maximum AlAT activity was shown by adult females among the different stages of life cycle. Our results are consistent with the results obtained in bruchid, *Zabrotes subfasciatus* (Kaur, 1985) and in *Drosophila* (Schneider and Chen, 1981). To the contrary, AsAT activity was maximum in the first instar of *C. quinquefasciatus,* declined significantly up to pupal stage and elevated again in adult males and females. These results agreed with the observation made by Evans and Kaleysaraj (1992) in *C. quinquefasciatus*. The ratio of AlAT/AsAT, which is the index of transamination and nitrogen balance in lower group of organisms (Nayar and Evans, 2011) and an index of liver function in mammals (Subramoniam *et al*., 1998) and that in *C. quinquefasciatus* first instar to pupa had a ratio $\leq$ 0.3. Elevation in AlAT/AsAT ratio shown by adult males and females (i.e. 0.6) can be considered as an adaptation of aerial life. Increase in the activity of transaminases in adults compared to larval instars and elevated AlAT/AsAT ratio also have been observed in *Oryctes rhinoceros* (Nayar and Evans, 2011) and in *Oecophylla smaragdina* (Vidhu and Evans, 2011). During early adult life AlAT and AsAT are still active, as the early part of adult life of insect is physiologically quite vigorous and energy requirements of the body at this stage are intense. The most important physiological functions of transaminases are the maintenance of an amino acid pool for protein synthesis (Meister, 1965), the

supply of metabolites for energy metabolism (Sacktor, 1974) and the catalysis of interactions between protein and carbohydrate metabolism (Katanuma *et al*., 1968).

During larval growth and pharate - adult development, the high AsAT activity involves the synthesis of proteins which subsequent to their participation in the formation and differentiation of larval and adult structures get transformed in to insoluble structural proteins (Pant and Kumar, 1980). This leads one to conclude that during growth and differentiation (histogenesis) the aminotransferases are highly active, while during histolysis, it is the reverse. Elevation of transaminase activity (AlAT/AsAT ratio) in adult *C. quinquefasciatus* may be related tothe aerial life of adults which necessitated the increased turnover of metabolites than the larvae.

Gradual decrease in the activity of glutamate dehydrogenase (GDH) from 1st instar up to pupa, together with a steep elevation in GDH activity in non- blood fed newly hatched female and male mosquitoes, can be correlated with their physiological states. Through the decrease in GDH activity, the biological availability of alpha-ketoglutarate formed from glutamate is restricted so that shuttling of all glucogenic amino acids into Krebs cycle is also restricted. This will help the fourth instar larvae to conserve amino acids for future use to synthesize new proteins. Adult females showed more GDH activity than adult males.In all larval stages, pupae and adults there exhibited a close relation between AsAT and GDH activity. It has been reported that the AsAT and GDH activity are closely related and transaminase – dehydrogenase complex is necessary in insects for the continuous supply of glutamate (Bursell,1970). Glutamate is the most abundant amino acid of free amino acid pool, playing significant roles as neurotransmitter, energy releaser and also as an amino acid responsible for acid- base balance of the haemolymph (Chen, 1966).

In the present investigation, activity of phenol oxidase (PO) showed a sharp, significant elevation from 1st instar larvae to emerging adults. Adult females showed the maximum PO activity. However, the activity of PO declined significantly after 48 hours of emergence. Our findings agree very well with the findings in red turpentine beetle, *Dendroctonus valens* (Shi *et al*., 2010). PO is an oxidoreductase produced in inactive prophenol oxidase form, and catalyzes the oxidation of phenols to quinines, which then polymerize non-enzymatically to produce melanization (Sugumaran, 1996). Melanization involves a series of diet-dependent chemical reactions involved in cuticle pigmentation, moulting, tissue repair and defense against pathogens (Gillespie *et al*., 1997, Rolf and Siva- Jothy, 2003; Schmid- Hempel, 2005; Siva-Jothy *et al*., 2005) In many species, the degree of cuticle melanization is a strong indicator of resistance to pathogens and is correlated with PO activity in the cuticle, haemolymph and midgut (Barnes and Siva- jothy, 2000; Wilson *et al*., 2001). The level of active PO seems to be correlated with the degree of pigmentation of the cuticle during stages of development (Giglio and Giulianini, 2013).

The first instar *C. quinquefasciatus* larvae are almost transparent and the activityof PO measured is very low. From 2nd instar larvae to pupa, gradual darkening of cuticle was observed and this increase in pigmentation and sclerotisation may have an adaptive advantage in decreasing the ability of fungal and bacterial proteases of polluted aquatic ecosystems. Newly hatched pharate adults are with fragile flimsy body, which gradually hardens and darkens within 2 days. Elevated ability of PO may facilitate the newly hatched mosquitoes to lead a successful winged life by providing a hard structural frame to the body. However, after 48 hours of adult emergence, cuticular melanization and sclerotization seems to be completed and hence there is a decline in activity of phenoloxidase.

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Two new records of Lithosiini (Lepidoptera: Erebidae: Arctiinae) from India

Navneet Singh[1] *, Rahul Joshi[2] and Jalil Ahmad[3]

[1] Zoological Survey of India, Kolkata 700053, West Bengal, India.
[2,3] Zoological Survey of India, Patna 800 026, Bihar, India.
Email: nsgill007@gmail.com; joshiarctiidae@gmail.com; jalilahmad23046@gmail.com

ABSTRACT: Two Lithosiin species, *Dolgoma rectoides* Dubatolov, 2012 and *Microlithosia pseudodecreta* Bucsek, 2012 are reported from India. Diagnosis, photographs of adults and male genitalia of both the species are provided. © 2018 Association for Advancement of Entomology

KEYWORDS: Arctiinae, Lithosiini, *Dolgoma rectoides, Microlithosia pseudodecreta,* new records, India

INTRODUCTION

The tribe Lithosiini of subfamily Arctiinae (Erebidae) is known by an estimated number of 3445 species in the World, with 909 species from Oriental region (Heppner, 1991) and 387 species from India (Singh *et al.,* 2014; Kirti & Singh, 2015, 2016; Joshi *et al.,* 2015, 2015a, 2016, 2017; Singh *et al.,* 2017, 2017a). Majority of the Lithosiin species are lichen feeders and therefore, act as important environmental indicators of air-pollution as lichens are sensitive to different types of air pollutants. Therefore, the study of Lithosiin diversity, distributional ranges or limits of these species will help in assessing environmental health and zoogeographical affinities of a particular area. This paper deals with addition of two Lithosiin species to Indian fauna: *Dolgoma rectoides* Dubatolov and *Microlithosia pseudodecreta* Bucsek, earlier recorded from Vietnam and Malaysia, respectively.

MATERIALS AND METHODS

Adult moths were collected with the help of vertical sheet light trap. The specimens collected were killed using ethyl acetate vapors and processed as per standard techniques in Lepidopterology. Dry preservation is done in fumigated entomological boxes and registered in the National Zoological Collections of ZSI, Gangetic Plains Regional Centre, Patna.

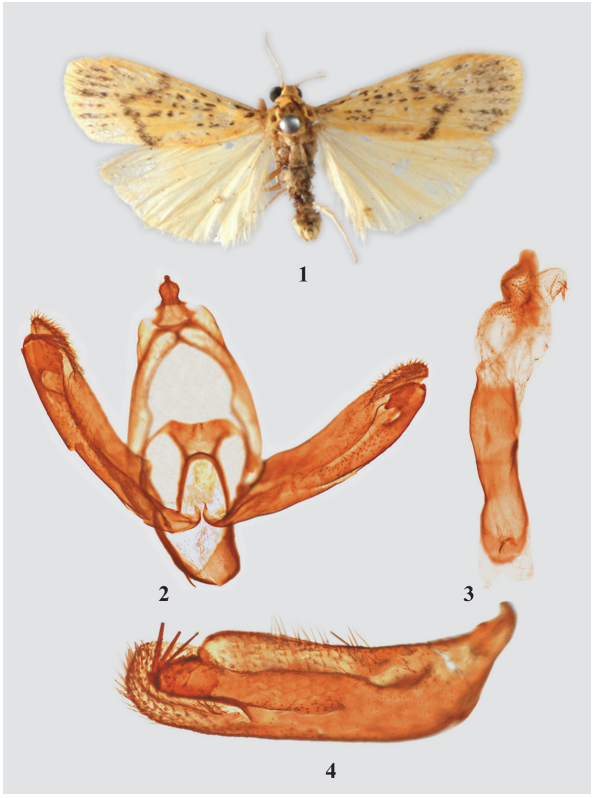
RESULTS AND OBSERVATIONS

Genus *Dolgoma* Moore, 1878

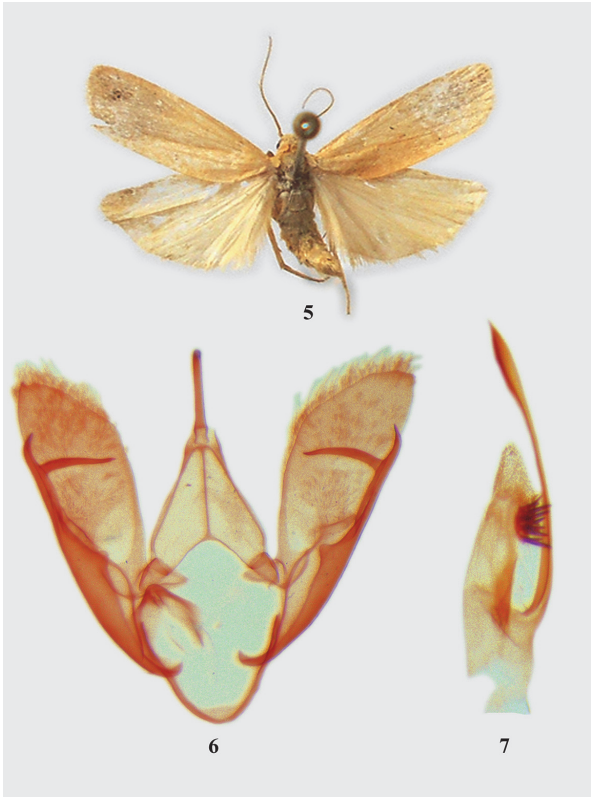
Proc. Zool. Soc. London **1878**: 20.

Type species: *Lithosia reticulata* Moore, 1865 (by original designation).

* Author for correspondence



FIGURES: 1, 2, 3, 4. *Dolgoma rectoides* Dubatolov: 1. Adult moth, 2. Male genitalia, 3. Aedeagus, 4. Valvae from inner side



FIGURES: 5, 6, 7. *Microlithosia pseudodecreta* Bucsek: 5. Adult moth, 6. Male genitalia, 7. Aedeagus.

Dolgoma rectoides Dubatolov, 2012 (Figs. 1, 2, 3, 4)

Dolgoma rectoides Dubatolov, 2012; *Euroasian Entomological Journal,* 11(6): 507-512

Material examined: INDIA: Arunachal Pradesh, Ziro, 1♂, 28.viii.2005; Coll. Singh, N.; INDIA: Arunachal Pradesh, Ziro, 2♂, 02.iv.09; Coll. Joshi, R.

Distribution: India (Arunachal Pradesh) (New record); Vietnam.

Taxonomic note: The adults are yellow, dusted with diffuse black dots, densely placed in basal and outer area of forewing; latter with a medial line, strongly angled outward at vein M_3. Male genitalia with valve having cucullus apex broad, rounded, covered with fine hairs; distal saccular process bears three long and strong downwardly directed spines.

Remarks: At present the genus *Dolgoma* is known by 15 species from the world and five species: *D. reticulata* Moore, *D. angulifera* (Felder), *D. oblitterans* (Felder), *D. xanthocraspis* (Hampson), and *D. recta* Černý are known from India. *D. rectoides* is the sixth species of *Dolgoma* from India. It is very similar to D. *recta* and *D. angulifera*, but can be distinguished from the two species as well from all its other congeners by the presence of three long and strong downwardly directed spines at the apex of sacculus.

Genus *Microlithosia* Daniel, 1954

Bonn. Zool. Beitr. 5 (1-2): 135.

Type species: *Microlithosia shaowuica* (by monotypy).

***Microlithosia pseudodecreta* Bucsek, 2012 (Figs. 5, 6, 7)**

Microlithosia pseudodecreta Bucsek, 2012; *Erebidae, Arctiinae (Lithosiini, Arctiini) of Malay Peninsula – Malaysia,* 2012: 136.

Material Examined: INDIA: Bihar, East Champaran, Valmiki Tiger Reserve (VTR), Bhediyari gate, 4♂, 09.x.2017; VTR, Naurangia Don, 4♂, 13.x.2017; VTR, Govardhana, 1♂, 14.x.2017; Coll. Singh, N. & Ahmad, J.

Distribution: India (Bihar) (New Record); Malaysia.

Taxonomic note: The adults are ochreous with forewing light brown, basal portion lighter in coloration, underside with broad black patch at termen, hindwing straw yellow. Male genitalia with valve having basal saccular process reduced to a knob like structure; cucullus triangular at apex; vesica with single group of large spines.

Remarks: Genus *Microlithosia* is readily diagnosed by a long process originating from the aedeagus. The process having its apex covered with small spines. The genus is known by seven species from the world, with two - *M. champhaiensis* Singh & Kirti, 2016 and *M. jagbiri* Singh & Joshi, 2017 from India. *M. pseudodecreta* is the third species from India and is closely similar to *M. decreta* but is distinct due to the shape of the distal saccular process and ornamentation of spines in vesica.

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Systematic survey on alpha diversity of anthophilous insect fauna in Binsar Wildlife Sanctuary, Western Himalaya

Manoj Kumar Arya*, Prachi Tamta and Aman Verma

Department of Zoology, D.S.B. Campus, Kumaun University, Nainital 263002 Uttarakhand, India. Email: dr.manojkumar19@rediffmail.com; amannaini92@gmail.com

ABSTRACT: Insects as pollinators or anthophiles are key components for proper functioning and long term sustainability of the agro and forest-ecosystems. Investigations undertaken to determine the status and diversity of insect pollinators in relation to the floristic composition in Binsar Wildlife Sanctuary (BWLS), Uttarakhand, India, revealed a total of 53 species of insects belonging to 18 families under four orders facilitating the pollination process in the entire area of the sanctuary. The species richness and value of Shannon Wiener diversity index (H') was recorded highest for the order Lepidoptera i.e., 33 species and 3.064, followed by Hymenoptera (11 species and 2.233), Diptera (five species and 1.495) and Coleoptera (four species and 1.226), respectively. The members of order Hymenoptera were much more evenly distributed with highest 0.9313 value of Pielou's Evenness Index (J') in comparisons to the other orders throughout the study period. In addition, the three study sites which were selected in the BWLS exhibited a declining trend of pollinators' alpha diversity along increasing altitudes. In the present study several plant species of families Asteraceae, Fabaceae, Rosaceae and Urticaceae constituted important foraging resources for insects throughout the years. Temporal variations in patterns of plant-pollinators interactions get affected by multiple environmental factors and different habitat types of BWLS, were also observed.

KEYWORDS: Altitudes, anthophiles, forest ecosystem, pollination, Western Himalaya.

INTRODUCTION

Despite the existing legal policies and regulations, India is facing a plethora of inter-related challenges such as degrading environmental quality, declining ecosystem services, deforestation, biodiversity loss, human wildlife conflict and climate change (Singh and Bagchi, 2013). The establishment of protected areas ensures not only the conservation of biodiversity but also maintain a wide range of ecosystem services for socio-economic and cultural well being (Stolton *et al*., 2015). However, studies suggest that local floral and faunal extinction, even in the protected areas of many developing countries can occur which is often linked with the anthropogenic pressures on the resources (Brashares, 2003; Pillay *et al*., 2011). Animals, insects in particular that visits flowering plants in order to obtain pollen, nectar, oils or floral

* Author for correspondence

tissues are considered as anthophiles or well known as pollinators (Kevan and Baker, 1983; 1998). Both domesticated and wild pollinator populations of insects are helpful in providing one of the most important ecosystem services of pollination crucial for the maintenance of wild plant communities, agricultural productivity and conservation and sustainability of global biodiversity (Kevan, 2003a; Ashman *et al*., 2004; Klein *et al*., 2007). It has been estimated that pollination of approximately 87% of all flowering plants are affected by animals (Ollerton *et al*., 2011) and moreover, about 80% of wild floral biodiversity is directly dependent on insect pollination for fruit and seed production with bees being the most efficient pollinators among other anthophilous insects (Ashman *et al*., 2004; Potts *et al*., 2010). Internationally, there is a grave concern over the widespread declining trends of pollination services in most of the ecosystems which have been disrupted by the multiple interactive effects such as changes in land use patterns, unusual climatic conditions, various environmental stresses, pesticide applications, spread of pests and pathogens, decreased resource diversity and others (Kevan, 2003a; Carvell *et al*., 2006; Winfree *et al*., 2009; Potts *et al*., 2010; Vanbergen and IPI, 2013). Thus, in the context of this, it becomes essential to evaluate plant-pollinators interactions for monitoring ecosystem functions and disruption (Senapathi *et al*., 2015) as well as for managing habitat for biodiversity (Gilgert and Vaughan, 2011). Many studies on insect pollinators in agricultural ecosystems of the world have been made by various workers which have contributed much to our understandings on pollination of agricultural and horticultural crops (Kevan, 1999; Raju and Reddi, 2000; Kevan, 2003b; Mishra *et al.,* 2004; Joshi and Joshi, 2010; Mattu *et al*., 2012; Raj *et al.*, 2012; Sharma and Mitra, 2012; Ganie *et al*., 2013; Raj and Mattu, 2014; Mattu and Bhagat, 2015; Kapkoti *et al.,* 2016; Khan *et al.,* 2016; Mattu and Bhagat, 2016). However, such studies on pollination services provided by insects in forest ecosystems are few (Campbell and Hanula, 2007; Winfree *et al*., 2007; Thakur and Mattu, 2010; Hussain *et al.,* 2012; Pandey *et al.,* 2013; Arya, 2015; Brunismann *et al.,* 2016). Moreover, pollination services of many wild plants have remained poorly understood (Potts *et al*., 2010). The present study, thus aims to highlight the importance of insect pollinator species of natural ecosystems in relation to their floral plants based on collections and observations in temperate forests of Binsar Wildlife Sanctuary located in the Western Himalayan region.

MATERIALS AND METHODS

(1) General description of the study area

The Himalayas in India account for more than 50 percent of its geographical area under forest cover and comprise 40 percent of species endemic to Indian subcontinent (Pandey *et al.,* 2013). Binsar Wildlife Sanctuary (BWLS) with a geographical area of 47.67 sq. km located between two districts of Uttarakhand state namely Almora and Bageshwar represents one of the oldest protected landscapes the Kumaun Himalayan region. Binsar is a fascinating spot that offers a majestic glimpse of the snow capped Indian Himalayan peaks namely Nanda Devi, Trishul and Panchachuli, presenting a unique experience to its visitors. The geographical location of Binsar Wildlife Sanctuary is 29^{o} 39'× 29^{o} 44' N and 79^{o} 41'× 79^{o} 49' E and the altitude varies between 1200 to 2500 meters above sea level. The sanctuary has core zone (4 sq. km) and buffer zone (43.67 sq. km). No human activity is allowed in Core Zone (Restricted Zone). Prior to India's independence in 1947, the study area was notified as "Protected Forest'' in 1893 and later upgraded as "Reserve Forest" in 1897. After independence, its status was revived to "Wildlife Sanctuary" by the Government of India in the year 1988. The climatic conditions prevailing in the BWLS range from temperate to sub-temperate type. Binsar represents the characteristic floral element of moist temperate type of vegetation.

For the present study, three study sites were selected in the Binsar Wildlife Sanctuary in a manner that they represented different altitudes and vegetation type (Table 1). A total of 25 species of trees, 34 species of shrubs and 55 species of herbs were recorded from different study sites of the protected area. Fig. 1 shows the graph of relative numbers of plant diversity recorded from different study sites of BWLS and the description of each study site is as under:

Site-1 (**Ayarpani):** This site is located adjacent to the main highway of Almora-Bageshwar. It is about 35 kms north of Almora town in Uttarakhand. It is the entry gate for the sanctuary and because of its proximity to highway, the area nearby it receives high level of disturbances due to tourism, transportation activities and other associated anthropogenic pressures. During the study period, temperature of this study site varied from 9ºC (January) to 29ºC (June), while the relative humidity ranged between 56% (November) to 88% (August).

Site-2 (Binneshwar Mahadev): This site is located near the Binneshwar temple within Binsar Wildlife Sanctuary and approximately 13 kms away from main highway (Almora-Bageshwar). This site receives moderate level of disturbance due to animal grazing, collection of minor and major forest products by neighbouring villagers and tourism. March and April are the months when flowers, especially ruby red *Rhododendron*, are in full bloom. The oak habitat is moderately dense and diverse in comparison to the pine habitat (Site-1). During the study period, temperature of this study site varied between 8.3ºC to 27ºC, while the relative humidity ranged between 57% (November) to 89% (August).

Site-3 (Jhandi Dhar): This study site is perched at an elevation of 2450 meters above mean sea level on the Jhandi Dhar hills, and is one of highest hill tops in the Kumaun region. This part of study area is also known as 'Zero Point' in BWLS providing scenic panoramic view of the Himalayan peaks. This site receives very low level of disturbances. This is highly snow prone area of the sanctuary receiving snow from mid of December till the mid of March. The habitat is denser but less diverse in comparison to the Site-2. During the study period, temperature of this study site varied between 6ºC (January) to 26ºC (June), while the relative humidity ranged between 57.8% (November) to 90% (August).

(2) Taxonomic survey and analysis of anthophilous insect fauna

Multiple samplings were carried out at an interval of 30 days by direct observations on either side of transects mainly during 08:00-11:00 and 03:00-05:00 hours of a day from July, 2013 to June, 2015 in order to assess diversity and abundance of anthophilous insect fauna at three different study sites of BWLS. The collection of species of insects belonging to different orders was made by hand picking, net sweeping and aspirator methods (Gadgkar *et al*., 1990 and Jonathan, 1990). The collected insects were preserved, identified using insect identification guides and by the scientists in Entomological Division of Forest Research Institute, Dehradun, India and later sorted taxonomically into different families and orders to prepare an inventory of anthophilous insect fauna of BWLS. Status to individual species was assigned as Very Common (VC) when counted in large numbers, Common (C) when observed regularly, Uncommon (UC) when recorded occasionally and Rare (R) when recorded rarely. Lastly, the collected data on pollination was analysed statistically using the program PAST (2005) in order to determine various measures of alpha diversity.

RESULTS AND DISCUSSION

During the present systematic survey on anthophiles of temperate region, a total of 2177 individuals of 53 species under 18 families belonging to four orders of classs Insecta were counted in the activity of pollination across the

Table 1. Characteristic features of the study sites at Binsar Wildlife Sanctuary (BWLS)

Study sites	Altitude (m) above sea level	Geographical Co-ordinates	Dominant plant species
Site-1 Ayarpani	1757m	N-29°40.255' E-79°42.325'	*Pinus roxburghii*, *Pyrus pashia*, *Myrica esculenta*, *Quercus leucotrichophora*, *Viburnum continifalium*, *Viburnum mullah*, *Eupatorium adenopharum*, *Bergenia ciliate, Carex condensata*, *Arisaema propinquum* and *Trifolium repens*.
Site-2 Binneshwar Mahadev	2191m	N-29°41.965' E-79°44.950'	*Quercus semecarpifolia, Quercus floribunda, Aesculus indica, Rhododendron arboreum, Ainsliaea aptera, Artemisia nilagirica, Conyza javanica, Gallium elegens, Bistorta amplexicaulis, Circium arvense, Chrysopogon gryllus and Cynotis vaga.*
Site-3 Jhandi Dhar	2450m	N-29°42.443' E-79°45.254'	*Cedrus deodara, Quercus semecarpifolia, Quercus glauca, Quercus floribunda, Quercus leucotrichophora, Inula cuspidate, Myrsine africana, Rubus paniculatus, Artemisia nilagirica, Arundinella nepalensis, Calamintha umbrosa, Conyza japonica and Cynoglossum dentculatum.*

different study sites of BWLS (Table 2). Of these, maximum number of species belonged to the order Lepidoptera (33 species), followed by Hymenoptera (11 species), Diptera (five species) and Coleoptera (four species), respectively. The order Lepidoptera was found to be most abundant pollinator group with 64.03% of total individuals recorded, followed by Hymenoptera (15.02%), Coleoptera (13.55%) and Diptera (7.4%), respectively (Fig. 2). Such variations in dominance among insect community with Lepidoptera being predominant pollinator group also support to earlier findings from the different ecosystems of Western Himalaya (Joshi and Joshi, 2010; Pandey *et al*., 2013; Arya, 2015). However, studies that were confined to temperate fruit orchards have reported order Hymenoptera and Diptera of prime significance (Mattu *et al*., 2012; Raj *et al*., 2012; Sharma and Mitra, 2012; Ganie *et al*., 2013; Raj and Mattu, 2014; Mattu and Bhagat, 2015; Kapkoti *et al*., 2016). Table 3 shows variation in the pattern of alpha diversity indices among different orders of anthophilous insects during the entire study period. The value of Shannon Wiener Diversity index (H') was calculated as 3.608 for overall samplings of insect assemblage depicting a rich diversity of insect pollinators in the protected area.

Based on observations a highest number of 29 species belonging to different orders were categorized as uncommon during the study period whereas, species of the order Lepidoptera such as *Aglais cashmiriensis* Kollar, *Vanessa indica* Herbst, *Pieris canidia indica* Evans and *Coccinella septumpunctata* Linnaeus of the order Coleoptera were recorded as very common and most abundant species across different study sites. On the other hand, seven species of Lepidoptera, three species of Hymenoptera, two species each of Diptera and Coleoptera were found common. Apart from this, species such as *Acraea issoria anamala* Kollar, *Aulocera padama* Kollar, *Lasiommata schakra schakra* (Kollar), *Pontia daplidice* (Linnaeus) of the order Lepidoptera and *Salius flavus* Fabricius, *Scolia venusta* Smith of the order Hymenoptera were recorded as rare species in the sanctuary.

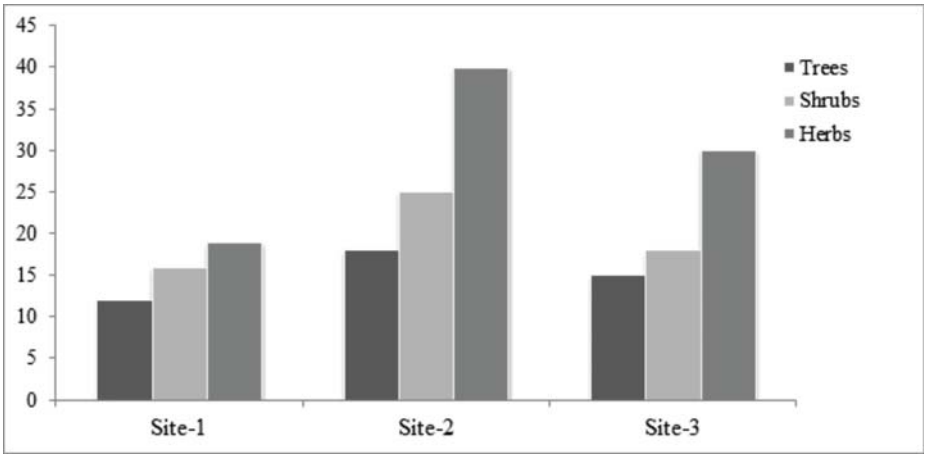

Fig. 1. Graph showing relative numbers of plant diversity recorded from different study sites of BWLS

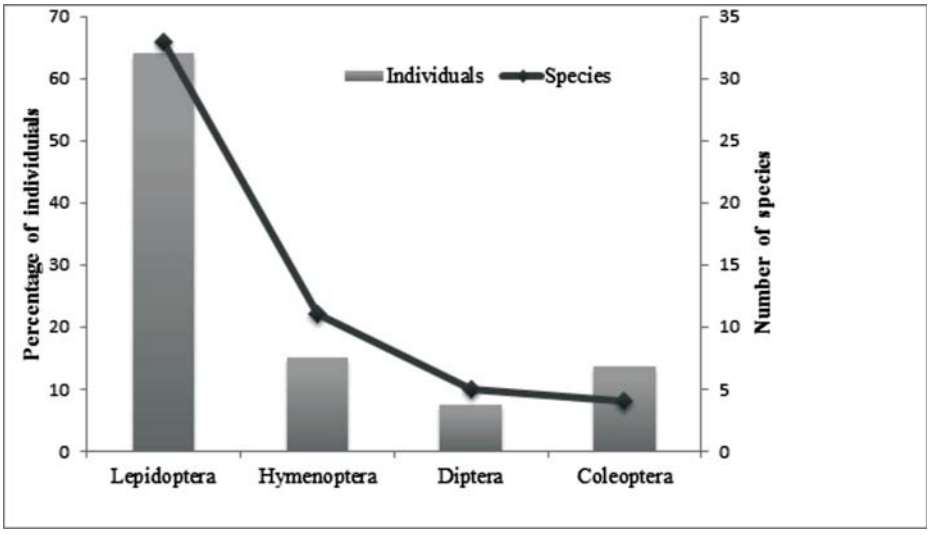

Fig. 2. Graph of species richness and abundance of different insect orders recorded as anthophiles during 2013-15

In the temperate regions the temporal variation in abundance and activity of herbivorous insects varies substantially, as related in response to more dramatic changes in weather conditions such as temperature maxima and minima, sunshine hours and rainfall then in the tropics with moderately constant environment (Wolda, 1988; Nestel *et al*., 1994; Speight *et al*., 1999). During the present study, out of total recorded species, 43 species were found in Site-1, followed by 41 species in Site-2 and 34 species in Site-3, respectively. Data in Table 4 shows the various measures of alpha diversity calculated for insect pollinator's community across different study sites. In general, the values of Shannon Wiener Diversity index (H'), Margalef's Diversity index (D) and Simpson's Dominance index varied significantly across the study sites showing decreasing trends in diversity, species richness and dominance of certain species of insect pollinators in response to variations in altitude (Rahbek, 1995; Malo and Baonza, 2002; Medan *et al*., 2002), habitat types and quality (Steffan-Dewenter and Tscharntke, 1999; Devoto *et al*., 2005; Kovas-Hostyanszki *et al.,* 2014). Moreover, the

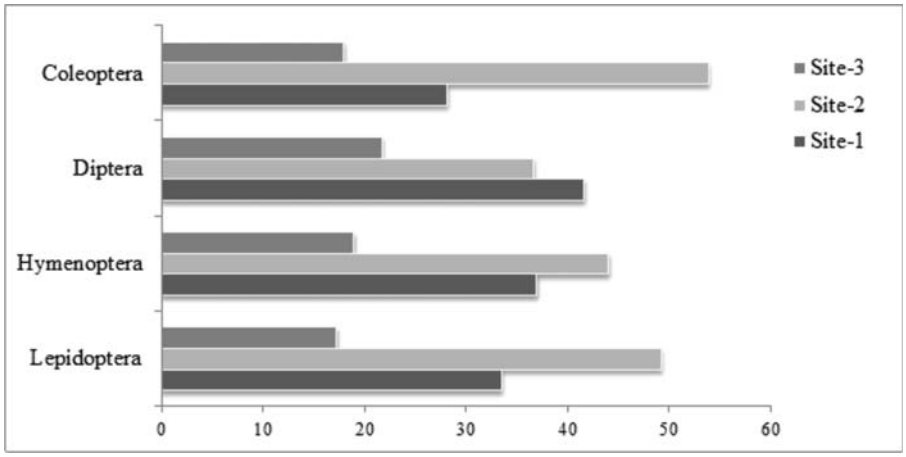

Fig. 3. Graph showing relative percentage composition of individuals of each order at different study sites

percentage of abundance of representative species and Pielou's Evenness index (J') was recorded highest for Site-2 i.e., 48.14% and 0.934, respectively expressing more even distribution of anthophilous insects. This might be attributed to efficient sharing of resources by each species as maximum number of plants was reported from Site-2 as compared to Site-1 and Site-3.

Fig. 3 indicates the relative percentage composition of individuals of each order at different study sites, wherein order Diptera shows a decreasing pattern of abundance with increasing altitudes of study sites. This is in slight contradiction with previous studies which suggests more abundance of flies at higher altitudes (Arroyo *et al*., 1982; Kearns, 1992; Devoto *et al*., 2005). However, the other three orders were abundant at Site -2, followed by Site-1 and least in Site-3, thus favoring the prevalence of more heterogeneous environment at Site-2. Overall, least abundance of insect assemblage at Site-3 might be due to more humid and wetter environment of the region which shows the least diversity of flower visiting taxa (Devoto *et al*., 2005). Out of total recorded species, 43.39% species have been recorded from all study sites in BWLS whereas 20.75% species were found restricted to only a single study site and hence can be considered as unique species. Species like *Scolia venusta* Smith of the order Hymenoptera and *Tabanus orientis* Walker of the order Diptera were reported only in the study Site-1. Species belonging to the order Lepidoptera such as *Danaus chryssippus* (Linnaeus), *Pseudoergolis wedah* (Kollar), *Phalanta phalantha* (Drury), *Anapheis aurata aurata* (Fabricius), *Eurema laeta laeta* Boisduval and those belonging to the order Hymenoptera like *Vespa* sp. and *Xylocopa fenesrata* Fabricius were restricted only to study Site-2. Such habitat specificity can be directly linked with the ecological demands of species such as the availability of host plants, atleast in the case of Lepidoptera (Thomas, 1995; Khan *et al*., 2011).

Patterns of plant-pollinators interactions may vary from species to species, with few species of insects are highly specialized while most of them show high degree of generalization (Faegri and Van-Der-Pijl, 1979). In the present study, members of plant families Asteraceae, Fabaceae, Rosaceae and Urticaceae and more importantly species like *Cirsium verutum*, *Circium arvense*, *Erigeron bonasiensis*, *Trifolium repens*, *Trifolium indicum* and *Pelia scripta* constituted important foraging plants throughout the years. Being predominant, the order Lepidoptera formed potential pollinator group among recorded

Table 2. Distribution pattern and relative abundance of different identified species of class Insecta recorded as anthophiles from BWLS

Sl. No.	Species Composition	Distribution at study areas			Relative Abundance	Status
		Site-1	Site-2	Site-3		
	Order: Lepidoptera					
	Family: Nymphalidae					
1.	*Acraea issoria anamala* Kollar	+	+	-	0.32	R
2.	*Aglais cashmiriensis* Kollar	+	+	+	8.45	VC
3.	*Argyreus hyperbius* Johanssen	+	-	+	0.96	UC
4.	*Aulocera padama* Kollar	-	-	+	0.04	R
5.	*Aulocera swaha swaha* Kollar	+	+	+	2.15	C
6.	*Danaus chryssippus* (Linnaeus)	-	+	-	0.64	UC
7.	*Euploea core* (Cramer)	+	+	-	1.28	UC
8.	*Kallima inachus* Boisduval	+	+	-	0.50	UC
9.	*Lasiommata schakra schakra* (Kollar)	+	+	-	0.32	R
10.	*Pseudoergolis wedah* (Kollar)	-	+	-	0.78	UC
11.	*Phalanta phalantha* (Drury)	-	+	-	1.10	UC
12.	*Sephisa dichroa* (Kollar)	+	-	+	0.50	UC
13.	*Vanessa cardui* Linnaeus	+	+	+	1.05	UC
14.	*Vanessa indica* Herbst	+	+	+	5.51	VC
	Family: Pieridae					
15.	*Anphaeis aurata aurata* (Fabricius)	-	+	-	1.15	UC
16.	*Catopsilia pomona* Linnaeus	+	+	-	4.08	C
17.	*Colias electo fieldi* Menetries	+	-	+	1.98	C
18.	*Eurema brigitta rubella* Wallace	+	+	-	1.83	UC
19.	*Eurema hecabe* Linnaeus	+	+	+	3.50	C
20.	*Eurema laeta laeta* Boisduval	-	+	-	0.82	UC
21.	*Gonepteryx rhamni nepalensis* Linnaeus	+	+	+	2.94	C
22.	*Metaporia agathon* (Gray)	+	+	+	0.91	UC
23.	*Pieris brassicae* Linnaeus	+	+	+	4.40	C
24.	*Pieris canidia indica* Evans	+	+	+	8.13	VC
25.	*Pontia daplidice* (Linnaeus)	-	-	+	0.13	R
	Family: Lycaenidae					
26.	*Heliophorus sena* Kollar	+	+	+	1.83	UC
27.	*Lycaena pavana* (Kollar)	+	-	+	0.78	UC
	Family: Papilionidae					
28.	*Byasa polyeuctes* Doubleday	+	+	+	0.82	UC
29.	*Papilio polyctor* Boisduval	+	+	+	1.42	UC
30.	*Papilio polytes romulus* Linnaeus	+	+	+	2.11	C
	Family: Arctiidae					
31.	*Ceryx imaon* Cramer	+	+	-	1.60	UC
	Family: Noctuidae					
32.	*Calpe ophideroides* Guen.	+	-	+	0.64	UC

	Family: Sphingidae					
33.	*Macroglossum* sp.	+	-	+	1.24	UC
	Order: Hymenoptera					
	Family: Apidae					
34.	*Anthophora confusa* Smith	+	-	+	0.96	UC
35.	*Apis laboriosa* Smith	+	+	-	1.28	UC
36.	*Bombus haemorrhoidalis* Smith	+	+	-	2.52	C
37.	*Bremus* sp.	+	+	+	1.42	UC
38.	*Crocisa ramosa* Lepeletier	+	+	+	1.93	C
39.	*Coelioxy* sp.	+	+	+	1.10	UC
	Family: Scoliidae					
40.	*Compsomeris asiatica himalaya* Bar.	+	+	+	3.07	C
41.	*Scolia venusta* Smith	+	-	-	0.42	R
	Family: Pompilidae					
42.	*Salius flavus* Fabricius	-	+	+	0.42	R
	Family: Vespidae					
43.	*Vespa* sp.	-	+	-	1.10	UC
	Family: Xylocopidae					
44.	*Xylocopa fenestrata* Fabricius	-	+	-	0.78	UC
	Order: Diptera					
	Family: Tabanidae					
45.	*Pangonia longirostris* Hardwicke	+	+	-	2.06	C
46.	*Philoliche* sp.	+	-	+	0.55	UC
47.	*Tabanus orientis* Walker	+	-	-	0.82	UC
	Family: Syrphidae					
48.	*Syrphus fulvifacies* Brunetti	+	+	+	2.25	C
	Family: Tipulidae					
49.	*Tipula himalayensis* Brunetti	+	+	+	1.70	UC
	Order: Coleoptera					
	Family: Chrysomelidae					
50.	*Altica himensis* Shukla	+	+	+	4.18	C
	Family: Coccinelidae					
51.	*Coccinella septumpunctata* Linnaeus	+	+	+	5.60	VC
	Family: Meloidae					
52.	*Mylabris cichorii* Linnaeus	+	+	+	2.98	C
53.	*Mylabris* sp.	+	+	+	0.78	UC

anthophiles during the present study. Species like *Aulocera swaha swaha* Kollar, *Vanessa cardui* Linnaeus, *Pieris canidia indica* Evans and *Byasa polyeuctes* Doubleday were foraging on *Aesculus indica* (Indian horse-chestnut) while species such as *Aglais cashmiriensis* Kollar, *Vanessa indica* Herbst, *Eurema hecabe* Linnaeus, *Colias electo fieldi* Menetries and *Papilio polytes romulus* Linnaeus were found frequent on plant species like *Cirsium verutum*, *Circium arvense*, *Eupatorium adenopharum*, *Gallium rotundifolium* and *Urtica dioca*. Species such as *Aulocera padama* Kollar, *Anphaeis aurata aurata* (Fabricius) and

Table 3. Variation in the pattern of alpha diversity among different orders of anthophilous insects in the BWLS

Diversity Indices	Lepidoptera	Hymenoptera	Diptera	Coleoptera	Total
Simpson	0.9361	0.8778	0.7584	0.6819	0.9639
Shannon	3.064	2.233	1.495	1.226	3.608
Margalef	4.42	1.727	0.7872	0.5275	6.766
Pielou/ Equitability	0.8764	0.9313	0.9288	0.8841	0.9088

Table 4. Variation in the diversity indices values of insects as calculated for different study sites in the BWLS

Diversity Indices	Site-1	Site-2	Site-3
Simpson	0.9626	0.9625	0.9457
Shannon	3.488	3.469	3.179
Margalef	6.36	5.752	5.529
Pielou/ Equitability	0.9274	0.9341	0.9016

Gonepteryx rhamni nepalensis Linnaeus were found nectaring on plant species namely, *Pyracantha crenulata*, *Anaphalis contorta* and *Salvia* sp., respectively. Species belonging to family Lycaenidae such as *Heliophorus sena* Kollar and *Lycaena pavana* (Kollar) were reported on *Fragaria daltoniana*, *Indigofera dosua*, *I. heterantha*, *Anaphalis cinnamonea* and *Circium arvense*. *Macroglossum* sp. of family Sphingidae was collected on *Arisaema propinquum* and moreover, family Sphingidae constitutes a major class of pollinator in many parts of the world (Frankie *et al*., 1983; Opler, 1983). Among Hymenoptera, members of the family Apidae are highly adapted and diverse anthophiles structurally, behaviorally and taxonomically (Kevan, 2003a). During the present study, *Bombus haemorrhoidalis* Smith, *Bremus* sp. and *Coelioxy* sp. were found on plants like *Berberis asiatica*, *Calamintha umbrosa*, *Digitalis purpurea*, *Inula cuspidate*, *Oxalis corniculata*, *Pyracantha crenulata*, *Rubus ellipticus*, *Rubus lasiocarpus*, *Rubus paniculatus*, *Bistorta amplexicaulis* and *Verbena officinalis* whereas species namely, *Anthophora cuspida* Smith and *Apis laboriosa* Smith were counted on plant species like *Lyonia ovalifolia*, *Pyrus pashia* and *Rhododendron arboretum*. Members of family Scoliidae, Pompilidae, Vespidae and Xylocopidae were collected on plants belonging majorly to families Araceae, Asteraceae, Geraniaceae and Fabaceae. In addition, species of *Xylocopa* are considered as important and most generalized pollinators in the natural ecosystems of the world (Raju and Reddi, 2000; Chamorro *et al*., 2012; Senapathi *et al*., 2015). In the present study, species like *Syrphus fulvifacies* of family Syrphidae belonging to the order Diptera were recorded on *Erigeron bonasiensis* and *Myrsine semiserrata* while species of the families Tabanidae and Tipulidae were found on *Berberis asiatica*, *Daphne papyracea*, *Deutzia staminea*, *Erythrina arborescens*, *Hypericum hookerianum*, *Inula cuspidate*, *Rubus ellipticus*, *Arisaema tortuosum*, *Bistorta amplexicaulis*, *Roscoea procera*, *Pyrus pashia* and *Sonchus oleraceus*. Similarly, species belonging to the order Coleoptera such as *Altica himensis* Shukla, *Coccinella septumpunctata* Linnaeus, *Mylabris cichorii* Linnaeus and *Mylabris* sp. were counted on

Circium arvense, *Cirsium verutum*, *Erigeron bonasiensis*, *Erythrina arborescens*, *Gallium elegans*, *Gallium rotundifolium*, *Geranium nepalense*, *Potentilla fulgens* and *Urtica dioca*.

The present study is the first of this type of study in the area of BWLS that might serve as a baseline data for future plant-pollinators relationships in the region. Our results suggest that rich assemblage of insects like butterflies, bees, wasps, flies and beetles constituted one among the primary groups of pollinators of biologically diverse wild plants with few species highly restricted to particular study sites. These species can be used to monitor environmental and climatic changes as they show a high degree of specialization and are critical to healthy and sustainable ecosystems. Moreover, the differences in the subsequent interactions of plant-pollinators may depend on several intrinsic and extrinsic factors that prevailed in the different habitats during the entire study period.

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Eco-friendly management of pod bugs of yard long bean (*Vigna unguiculata* sub sp. *sesquipedalis* (L.) Verdcourt) under field conditions

V. Vineetha* **and B. Ramesha**

College of Agriculture, Kerala Agricultural University, Padannakkad, Kasaragod, Kerala 671314, India. Email:vineethavenugopal72@gmail.com

ABSTRACT: The study on the eco-friendly management of pod bugs *viz.*, *Riptortus pedestris* (F.) (Hemiptera: Coreidae); *Clavigralla gibbosa* Spinola (Hemiptera: Coreidae); *Nezara viridula* (L.) (Hemiptera: Pentatomidae) of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) under field conditions was conducted during kharif and rabi seasons in the year 2016. Among the biopesticides treated, Azadirachtin 1% resulted in complete reduction of pod infestation by pod bugs even after fifteen days of second spray followed by *Lecanicillium lecanii* where complete reduction of pod bug infestation was noticed fifteen days after third spray.

KEY WORDS: *Vigna unguiculata* subsp. *sesquipedalis*, *Riptortus pedestris*, *Clavigralla gibbosa*, *Nezara viridula*, management, biopesticides, azadirachtin

INTRODUCTION

One of the key components of Indian agricultural production is the legumes, among which vegetable cowpea or yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) imparts a major contribution. Cowpea is popularly known as 'vegetable meat' because of its high protein content. It is a crop of high value which requires only fewer inputs. The most important constraint that reduces the production and productivity of vegetable cowpea is the insect pests. Among the insect pests of vegetable cowpea, the important and the destructive post flowering pests are the pod bugs *viz., Riptortus pedestris* (F.); *Clavigralla gibbosa* Spinola; *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae) and *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Jackai and Daoust, 1986). In Kerala, the nymph and adult population of *N. viridula* attains its peak during May- April and the population of nymphs of *R. pedestris* was high during May and adults of *R. pedestris* was on its peak during first and second fortnight of June (Bharathimeena *et al.,* 2008). The attack of pod sucking bug, *C. tomentosicollis* results in desiccation and shrivelling of pods prematurely and formation of half filled pods. During its peak infestation, more than 80 per cent of yield loss occurs (Singh *et al*., 1990). For the management of these pests, different chemical insecticides are available in the market with different modes of action. The inappropriate use of insecticides causes build up of resistance in target species, resurrection of other pest species, devastation of natural enemies, disarray of ecosystem and considerable health impacts (Khade *et al*., 2014). Taking into consideration of these

* Author for correspondence

issues, some viable environment friendly alternatives have to be found out especially in Kasaragod district as it has been under organic cultivation for the past five years. The lessons from the adoption of organic cultivation in Kasaragod district have been abstracted in the report of Menon (2015) which highlighted the need for studies with organic v/s insecticidal management in Kasaragod district.

The entomopathogenic fungi like *Beauveria bassiana* and *Metarhizium anisopliae* were reported as an important part of integrated pest management in cowpea (Srinivasan *et al*., 2009). The compounds of neem acts as insect growth regulator, oviposition repellent, inhibition of fecundity and antifeedant (Ascher, 1993). Spinosad 45 SC exhibits very low toxicity to mammals and no catastrophic effects on exposure for a long time (Gour and Sreedevi, 2012). With this view the present study aimed at studying the efficacy of different microbial agents, neem based and bio rational insecticides against pod bugs of yard long bean.

MATERIALS AND METHODS

The research work was carried out in the Instructional Farm of College of Agriculture, Padannakkad from May 2016 to August 2016 and September 2016 to December 2016 in RBD with 9 treatments and 3 replications @ twelve plants per treatment. The yard long bean variety 'Lola' released by KAU was selected for conducting the

Table 1. Mean per cent of pods infested by nymphs and adults of pod bugs taken at weekly intervals during kharif season from May to August 2016

Treatments	Mean per cent of infested pods					
	7 DAFS	15DAFS	7 DASS	15DASS	7 DATS	15DATS
T_1 - *Beauveria bassiana* @ 10^7 spores/ml	29.36 (5.51)	24.80 (5.08)	30.69 (5.63)	34.04 (5.92)	26.24 (5.22)	46.74 (6.91)
T_2 - *Metarhizium anisopliae* @ 10^7 spores/ml	40.99 (6.48)	46.19 (6.87)	88.11 (9.44)	70.23 (8.44)	49.55 (7.11)	66.40 (8.21)
T_3 – *Lecanicillium lecanii* @ 10^7 spores/ml	10.08 (3.33)	8.61 (3.10)	4.47 (2.34)	3.00 (2.00)	1.13 (1.46)	0.00 (1.00)
T_4 – *Bt* formulation @ 2× 10^8 cfu/ml @ 1 ml/l	42.42 (6.59)	46.47 (6.89)	43.22 (6.65)	38.43 (6.28)	25.62 (5.16)	40.60 (6.45)
T_5 – Neem (Azadirachtin 1%) @ 5ml/l	2.31 (1.82)	1.43 (1.56)	0.96 (1.40)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T_6 – Neem oil emulsion 5% 50ml/l	4.76 (2.40)	3.12 (2.03)	0.87 (1.37)	1.25 (1.5)	1.25 (1.5)	1.95 (1.72)
T_7 – Spinosad 45 SC @ 0.4 ml/l	30.47 (5.61)	25.31 (5.13)	24.30 (5.03)	31.37 (5.69)	38.31 (6.27)	33.81 (5.90)
T_8 – Malathion 50 EC @ 2ml/l	7.00 (2.83)	5.35 (2.52)	5.55 (2.56)	6.50 (2.74)	12.39 (3.66)	12.69 (3.70)
T_9 – Absolute control	49.55 (7.11)	58.13 (7.69)	87.73 (9.42)	91.16 (9.60)	76.96 (8.83)	83.82 (9.21)
C.D.(0.05 %)	1.57	2.2	1.90	1.79	1.99	1.40

Figures in parenthesis denotes $\sqrt{x+1}$ transformed values.

DAFS- Days after first spray; DASS- Days after second spray; DATS- Days after third spray.

Table 2. Mean per cent of pods infested by nymphs and adults of pod bugs taken at weekly intervals during rabi season from September 2016 to December 2016

Treatments	Mean per cent of infested pods					
	7 DAFS	15DAFS	7 DASS	15DASS	7 DATS	15DATS
T_1 - *Beauveria bassiana* @ 10^7 spores/ml	44.02 (6.71)	30.02 (5.57)	15.89 (4.11)	11.53 (3.54)	3.16 (2.04)	6.78 (2.78)
T_2 - *Metarhizium anisopliae* @ 10^7 spores/ml	51.56 (7.25)	53.90 (7.41)	44.15 (6.72)	25.21 (5.12)	24.70 (5.07)	19.34 (4.51)
T_3 – *Lecanicillium lecanii* @ 10^7 spores/ml	25.83 (5.18)	14.68 (3.96)	3.24 (2.06)	2.13 (1.77)	0.44 (1.20)	0.00 (1.00)
T_4 – *Bt* formulation @ 2×10^8 cfu/ml @ 1 ml/l	67.39 (8.27)	49.83 (7.13)	36.82 (6.15)	42.42 (6.59)	40.08 (6.41)	31.14 (5.67)
T_5 – Neem (Azadirachtin 1%) @ 5ml/l	3.24 (2.06)	1.95 (1.72)	0.87 (1.37)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T_6 – Neem oil emulsion 5% @ 50ml/l	17.74 (4.33)	14.13 (3.89)	6.89 (2.81)	4.01 (2.24)	3.92 (2.22)	5.45 (2.54)
T_7 – Spinosad 45 SC @ 0.4 ml/l	54.65 (7.46)	46.74 (6.91)	30.24 (5.59)	32.29 (5.77)	31.83 (5.73)	25.31 (5.13)
T_8 – Malathion 50 EC @ 2ml/l	0.00 (1.00)	4.61 (2.37)	1.59 (1.61)	13.06 (3.75)	3.53 (2.13)	3.36 (2.09)
T_9 – Absolute control	81.62 (9.09)	78.03 (8.89)	81.81 (9.10)	69.05 (8.37)	82.17 (9.12)	70.57 (8.46)
C.D.(0.05 %)	1.81	1.94	1.78	1.27	1.16	0.86

$\sqrt{x+1}$

Figures in parentheses denote transformed values.

DAFS- Days after first spray; DASS- Days after second spray; DATS- Days after third spray.

study. The crop was raised on trellis at a spacing of 1.5 x 0.45m. All the planting operations were done based on the Package of Practice recommendations: crops of KAU, 2016. The treatments included; T1- *Beauveria bassiana* (liquid formulation @ 10^7 spores/ml of water), T2- *Metarhizium anisopliae* (liquid formulation @ 10^7 spores/ml of water), T3- *Lecanicillium lecanii* (liquid formulation @ 10^7 spores/ml of water), T4- *Bt* formulation 2×10^8 cfu/ml @ 1 ml/l of water, T5- Neem based insecticide (Azadirachtin 1% @ 5 ml/l of water), T6- Neem oil emulsion 5% (50ml/l of water), T7- Spinosad 45 SC @ 0.4 ml/l of water, T8- Malathion 50 EC @ 2ml/l of water (standard check), T9- Absolute control.

The pure culture of entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* needed for the conduct of the research work were brought from National Bureau of Agricultural Insect Resources (NBAIR), Bangalore and were maintained throughout the period by sub culturing it on Potato Dextrose Agar medium (PDA) under laboratory conditions at regular intervals and mass multiplied on Potato Dextrose Broth (PDB). All the treatments were imposed at fortnightly intervals just after the initial attack of pest was seen and observations were recorded at weekly intervals corresponding to standard weeks by counting the number of nymphs/adults of pod bugs, number of infested pods out of total number of pods. The crop was harvested 60 days after planting. The data were subjected to square root transformation and analyzed using ANOVA.

RESULTS AND DISCUSSION

The efficacy of different entomopathogenic fungi, *Bt*, biorational and neem based insecticides against pod infestation by pod bugs during kharif season (May 2016 to August 2016) and rabi season (September 2016 to December 2016) are presented in the Table 1and 2. During kharif season, minimum per cent of pod infestation was noticed in T_5 (Azadirachtin 1%) with 2.31%, 1.43% and 0.96% infestation on 7th day after fist spray, 15th day after first spray and 7th day after second spray respectively. Thereafter no infestation on pods was noticed. Maximum infestation was noticed on T_9 with a range of 49.55 to 91.16% of pod infestation. Next to Azadirachtin, *Lecanicillium lecanii* (T_3) was effective in reducing the percentage of infestation after three consecutive sprays with a range of 10.08% on 7 days after first spray to 0.00% on 15 days after third spray. *L. lecanii* became on par with Azadirachtin only after fifteen days of third spray. This was followed by T_6 (neem oil 5%) which exhibited a minimum of 1.72% of infestation after three consecutive sprays (Table 1).

During rabi season, the percentage of pod infestation was found minimum in T_5 (Azadirachtin 1%) treated plot with 3.24%, 1.95% and 0.87% on 7th day after fist spray, 15th day after first spray and 7th day after second spray respectively. Complete reduction in pod infestation. T_5 followed by *L. lecanii* (T_3) having 0.44% infestation on 7 days after third spray and no infestation (0.00%) on 15 days after third spray. *L. lecanii* (T_3) was found to be on par with Azadirachtin 1% (T_5) only after fifteen days of third spray. Maximum infestation was noticed on T_9 with a range of 69.05 to 82.17% of pod infestation (Table 2).

Table 3. Effect of treatments on the yield attributes of yard long bean during kharif season from May 2016 to August 2016

Treatments	Fresh weight of pods (g/plant)				Total yield (g/plant)	Marketable yield (g/plant)
	First harvest	Second harvest	Third harvest	Fourth harvest	Total	Total
T_1 - *Beauveria bassiana* @ 10^7 spores/ml	69.03	94.40	107.25	128.46	399.14	377.16
T_2 - *Metarhizium anisopliae* @ 10^7 spores/ml	64.75	97.56	92.66	113.58	368.56	291.78
T_3 – *Lecanicillium lecanii* @ 10^7 spores/ml	85.45	97.83	109.16	108.27	400.73	346.43
T_4 – *Bt* formulation @ 2×10^8 cfu/ml @ 1 ml/l	58.99	67.19	100.08	117.63	343.89	323.19
T_5 – Neem (Azadirachtin 1%) @ 5ml/l	87.80	99.08	86.04	110.84	383.76	347.19
T_6 – Neem oil emulsion 5% @ 50ml/l	71.58	108.18	104.11	104.23	388.11	325.28
T_7 – Spinosad 45 SC @ 0.4 ml/l	83.78	145.75	123.33	131.01	483.88	466.46
T_8 – Malathion 50 EC @ 2ml/l	60.66	79.58	85.75	104.09	330.09	302.59
T_9 – Absolute control	63.58	78.30	91.83	89.74	323.45	237.17
C.D. (0.05 %)	17.47	15.54	14.13	12.62	30.02	35.33

Table 4. Effect of treatments on the yield attributes of yard long bean during rabi season from September 2016 to December 2016

Treatments	Fresh weight of pods (g/plant)							Total yield (g/plant)	Marketable yield (g/plant)
	First harvest	Second harvest	Third harvest	Fourth harvest	Fifth harvest	Sixth harvest	Seventh harvest	Total	Total
T_1 - *Beauveria bassiana* @ 10^7 spores/ml	17.25	35.33	39.08	41.50	90.83	331.31	137.40	692.71	629.13
T_2 - *Metarhizium anisopliae* @ 10^7 spores/ml	16.76	28.33	90.41	89.83	90.98	168.12	203.54	688.00	456.91
T_3 – *Lecanicillium lecanii* @ 10^7 spores/ml	20.62	27.31	83.62	74.77	101.66	151.69	143.08	602.78	580.72
T_4 – *Bt* formulation @ 2×10^8 cfu/ml @ 1 ml/l	6.00	23.45	72.66	59.90	43.66	166.66	63.65	436.00	410.37
T_5 – Neem (Azadirachtin 1%) @ 5ml/l	13.00	30.25	66.58	38.04	71.66	162.75	109.01	491.31	455.62
T_6 – Neem oil emulsion 5% @ 50ml/l	12.50	24.66	105.70	39.66	76.33	166.79	129.54	555.20	529.10
T_7 – Spinosad 45 SC @ 0.4 ml/l	24.30	41.00	144.25	75.04	117.00	191.74	145.40	738.74	718.24
T_8 – Malathion 50 EC @ 2ml/l	12.46	32.50	52.83	78.66	87.00	123.62	107.30	494.40	473.03
T_9 – Absolute control	19.16	28.35	40.00	55.96	39.33	77.50	60.00	320.31	249.25
C.D.(0.05 %)	5.38	6.57	16.04	26.42	31.12	48.12	33.81	47.73	54.92

Four harvests were done during kharif season and seven harvests were done during rabi season. During kharif season, from the total yield calculated, treatment T_7 recorded higher yield of 483.88 g per plant followed by T_3 with yield of 400.73 g per plant. Treatments *viz.,* T_3, T_1, T_6 and T_5 were statistically on par with each other recording 400.73, 399.14, 388.11 and 383.73 g per plant respectively. The total yield obtained was low in treatment T_9 (323.45 g per plant) followed by T_8 (330.09 g per plant). Highest marketable yield was also recorded in Treatment T_7 (466.46 g per plant) followed by T_1 with yield of 377.16 g per plant. Treatment T_9 recorded the lowest marketable yield of 237.17 g per plant. Treatments T_1, T_5 and T_3 were found to be on par with each other with 377.16, 347.19 and 346.43 g per plant respectively (Table 3).

During rabi season, from the total yield calculated, treatment T_7 recorded higher yield of 738.74 g per plant followed by T_1 (692.71 g per plant) and T_2 (688 g per plant). Thus T_1 and T_2 were statistically on par with T_7. Minimum yield was recorded in treatment T_9 with 320.31 g per plant. Treatments T_3 and T_6 were found on par with each other with 602.78 g and 555.20 g per plant respectively. Highest marketable yield was also recorded in Treatment T_7 (718.24 g per plant) followed by T_1 and T_3 with yield of 629.13 g per plant and 580.72 g per plant respectively. Thus treatments T_1 was found statistically on par with T_7. Treatment T_9 recorded the lowest marketable yield of 249.25 g per plant. Treatments T_3 and T_6 was found to be on par with each other having 580.72 g per plant and 529.10 g per plant respectively (Table 4).

During kharif season, maximum net returns were recorded in treatment T_7 (63250.00) followed by T_1 and T_3 with net returns 36249.80 and 23803.50 respectively. By applying treatment T_7, an amount

Table 5. Economics of cultivation of yard long bean during kharif season from May 2016 to August 2016

Treatments	Economics of yard long bean					
	Production cost excluding insecticides (Rs./ha)	Cost of insecticides (Rs./ha)	Total expenditure (Rs./ha)	Gross Income (Rs./ha)	Net income (Rs./ha)	B : C ratio
T_1 - *Beauveria bassiana* @ 10^7 spores/ml	115062.00	1440.00	116502.00	152751.80	36249.80	1.31
T_2 - *Metarhizium anisopliae* @ 10^7 spores/ml	115062.00	1440.00	116502.00	118172.30	1670.30	1.01
T_3 – *Lecanicillium lecanii* @ 10^7 spores/ml	115062.00	1440.00	116502.00	140305.00	23803.50	1.20
T_4 – *Bt* formulation @ 2×10^8 cfu/ml @ 1 ml/l	115062.00	1240.00	116302.00	130895.30	14593.30	1.12
T_5 – Neem (Azadirachtin 1%) @ 5ml/l	115062.00	2947.50	118009.50	140612.60	22602.63	1.19
T_6 – Neem oil emulsion 5% @ 50ml/l	115062.00	13500.00	128562.00	131739.80	3177.75	1.02
T_7 – Spinosad 45 SC @ 0.4 ml/l	115062.00	10607.00	125669.00	188919.00	63250.00	1.50
T_8 – Malathion 50 EC @ 2ml/l	115062.00	1350.00	116412.00	122549.60	6137.62	1.05
T_9 – Absolute control	115062.00	0.00	115062.00	96055.88	-61679.60	0.46

Table 6. Economics of cultivation of yard long bean during rabi season from September 2016 to December 2016

Treatments	Economics of yard long bean					
	Production cost excluding insecticides (Rs./ha)	Cost of insecticides (Rs./ha)	Total expenditure (Rs./ha)	Gross Income (Rs./ha)	Net income (Rs./ha)	B : C ratio
T_1 - *Beauveria bassiana* @ 10^7 spores/ml	115062.00	1440.00	116502.00	254799.00	138297.00	2.18
T_2 - *Metarhizium anisopliae* @ 10^7 spores/ml	115062.00	1440.00	116502.00	185051.30	68549.25	1.58
T_3 – *Lecanicillium lecanii* @ 10^7 spores/ml	115062.00	1440.00	116502.00	235193.60	118691.60	2.01
T_4 – *Bt* formulation @ 2×10^8 cfu/ml @ 1 ml/l	115062.00	1240.00	116302.00	166201.90	49899.88	1.42
T_5 – Neem (Azadirachtin 1%) @ 5ml/l	115062.00	2947.50	118009.50	184528.10	66518.63	1.56
T_6 – Neem oil emulsion 5% @ 50ml/l	115062.00	13500.00	128562.00	214288.90	85726.88	1.66
T_7 – Spinosad 45 SC @ 0.4 ml/l	115062.00	10607.00	125669.00	290887.90	162325.90	2.26
T_8 – Malathion 50 EC @ 2ml/l	115062.00	1350.00	116412.00	191578.50	65909.5	1.52
T_9 – Absolute control	115062.00	0.00	115062.00	100946.30	-15465.8	0.86

of Rs.1.5 was obtained for every one rupee invested against the treatment T9 which had a return of only Rs. 0.46. Treatment T_1 when applied earned a return of Rs. 1.31 for every one rupee invested. Treatment T_5 gave a return of Rs. 1.19 for every one rupee invested (Table 5).

During rabi season maximum net returns were recorded in treatment T_7 (162325.90) followed by T_1 and T_3 with net returns 138297.00 and 118691.60. Application of biorationals insecticide, Spinosad (T_7) gave a return of Rs. 2.26 for every one rupee invested. By applying treatment T_1, an amount of Rs.2.18 was obtained for every one rupee invested against the treatment T_9 which had a return of only Rs. 0.86. Treatment T_5 gave a return of Rs. 1.56 for every one rupee invested (Table 6).

Azadirachtin exhibited a drastic reduction in the per cent of pod damage even after two sprays and no pod damage was found after third spray which proved it to be the effective treatment. Azadirachtin helps in increasing the market value of the pods by reducing the pod damage. The findings of Koona *et al.* (2001) that with increase in the pod age the damage to the pods were minimized and the crucial period of infestation was seen in pods of eight days old was supporting to the present finding. Soyelu and Akingbhohungbe (2007) reported that greater reduction in the yield was caused by fourth instar nymphs of *Anoplocnemis curvipes, Riptortus dentipes, Mirperus jaculus* and *Clavigralla tomentosicollis*. The findings of Mordue and Nisbet (2000) that hemipterans are sensitive to high concentration of azadirachtin resulting in 100 per cent antifeedancy. Thus reducing the pod damage to a great extent was also a supporting fact. Next to Azadirachtin, another biopesticide which proved to be effective in controlling pod bugs was *L. lecanii* which reduced the percentage of infestation completely after three consecutive sprays. The findings of Suharsona and Prayago (2014) that *L. lecanii* @ 10^7 conidia/ml exhibited high degree of control on soyabean brown stink bug, *Riptortus linearis* in Indonesia was in line with the above study.

The total and marketable yield was found maximum in spinosad treated plot during both kharif and rabi season. The highest benefit-cost ratio was given by spinosad during both kharif and rabi seasons followed by *B. bassiana* treated plot. Spinosad though it is costly, high yield from spinosad treated plot could provide an additional amount than the amount invested which compensated the high cost of spinosad. The net returns were high for Spinosad during both seasons. Though *B. bassiana* encountered major pests, it didn't affect the yield severely during both seasons. The efficiency of bio pesticides in controlling insect pests without harming non-target species and its non-toxicity towards humans found to be the best approach among pest management strategies. Through this it is possible to increase good quality produce. Thus bio pesticides play a promising tool in pest management and are gaining prior importance in the present scenario.

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Taxonomic survey of four species of subfamily Apaturinae (Lepidoptera: Papilionoidea: Nymphalidae) from western Himalaya, India with illustration of external genitalia

Deepika Mehra*, Jagbir Singh Kirti, and Avtar Kaur Sidhu#

Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India; Email: deepika.mehra.nymphalidae@gmail.com; Email: prjagbir2005@gmail.com;
#Zoological Survey of India, High Altitude Regional Centre, Saproon, Solan, Himachal Pradesh, India. Email: avtarkaur2000@gmail.com

ABSTRACT: Four butterfly species viz., *Dilipa morigana* (Westwood), *Hestina persimilis* Butler, *Hestinalis nama* (Doubleday) and *Sephisa dichroa* (Kollar) under the subfamily Apaturinae from western Himalaya with external genitalic attributes were described and illustrated in detail. Pictorial identification keys for the taxa have been formulated.

KEYWORDS: Taxonomy, Apaturinae, external genitalia, western Himalaya

INTRODUCTION

Subfamily Apaturinae is one amongst the 12 subfamilies (Nymphalinae, Biblidinae, Calinaginae, Charaxinae, Cyrestinae, Danainae, Heliconiinae, Libytheinae, Limentidinae, Pseudergolinae, and Satyrinae) (Wahlberg *et al*., 2009) of the cosmopolitan butterfly family Nymphalidae (Lepidoptera) which includes about 7200 species occurring in all habitats and continents except Antarctica (DeVries, 1987; Shields, 1989; Harvey, 1991). The species of this subfamily are mostly dispersed in Eurasia, South-East Asia and Africa (Old World)while a very few species are distributed mainly in New world, indicating a disjunctive distributional pattern. The species under the subfamily Apaturinae are classified under 20 genera (Harvey 1991). Although the Apaturinae larvae mainly feed on the Cannabaceae, those of the genus *Apatura* are associated with *Salix* and *Populus* (Salicaceae), which are distantly related to the Cannabaceae (Ohshima *et al*., 2010).

In India, a total of 15 species referable to nine genera are found, whereas in western Himalaya, eight species referable to seven genera are found. The Apaturinae species are very fast and high fliers, fond of mud peddling, preferably sip from garbage exude and even from putrefying carcases of animals etc. Hence, some species can often be spotted near the garbage bins during the morning hours. The females of few species (e.g. *S. chandra* Moore) also exhibit polymorphism. Mimicry is also prominently advertised by both male and females of different species.

As compared to other subfamilies under Nymphalidae, the Apaturinae is relatively small and only scanty taxonomic information is available about the species distributed in India. The taxonomic

* Author for correspondence

characters help in constructing the higher classification of a group. So, in the present work, four species referable to four genera (*Dilipa morigana* (Westwood), *Hestina persimilis* Butler, *Hestinalis nama* (Doubleday), and *Sephisa dichroa* (Kollar)) under the subfamily Apaturinae have been taxonomically dealt with, and their external genitalic attributes have been illustrated in detail. Moreover, *Dilipa morigana* (Westwood) and *Sephisa dichroa* (Kollar) are the type species of their respective genera, and the former species is a Schedule-I species protected under Wild life (Protection) Act (1972).

MATERIALS AND METHODS

Collection-cum-survey tours were conducted to various localities in western Himalaya (Shivalik range and middle Himalaya) during the pre-monsoon and post monsoon season. Different ecosystems surveyed included: open grasslands, human inhabitations, subtropical evergreen forest above 1200m, tropical deciduous forest between 400 and 1400 m, cultivated lands, water shed areas and scrubland. The adult representatives of the family Apaturinae were actively collected by using the sweeping net. The collected butterfly specimens were killed with ethyl acetate vapours in the killing bottle. The specimens, while soft, were pinned and stretched on adjustable wooden stretching boxes, and were dried for 2-3 days. The dried specimens were preserved in air tight wooden boxes, containing naphthalene balls as fumigants. The specimens were photographed from the dorsal and ventral side, using a digital camera Nikon DSLR 3300 fitted with an 80 mm lens. To study the wing venation permanent slides of fore and hind wings were made by following the methodology provided by Common (1970) and advocated by Zimmerman (1978). For dissection and preparation of the external genitalia, the method proposed by Robinson (1976) was adopted. The terminology for the male genitalia has been adopted from Sibatani *et al*., (1954), Shirozu and Yamamoto (1956), Klots (1970), and Sibatani (1972).

The photography of the male and female external genitalia was accomplished by using Leica™ microscope equipped with a photographic unit. For the male genitalia, the right valve was removed with the help of the forceps and genitalia were photographed in a lateral and dorsal view (to highlight structure of uncus from dorsal side). The inner and outer view of the right valva was also photographed. For the female genitalia, generally lateral view was taken in consideration. The enlarged view of the signum (wherever present) has also been photographed. The reference material has been preserved in vial containing clove oil. Identification keys have been formulated for four taxa under consideration in this paper (Figure 1).

RESULTS AND DSCUSSION

Genus *Dilipa* Moore

Moore, 1857; in Horsfield & Moore, Cat. lep. Ins. Mus. East India Coy 1: 201.

Type species: *Apatura morgiana* Westwood

Westwood, [1850]; Gen. diurn. Lep. (2): 305 nota.

Body robust; head dressed with long hair; eyes prominent and glabrous; antennae equals to half costa, club well defined, compressed and long but gradual; labial palpi porrect, not extended beyond head; male genitalia with a very long saccus and aedeagus, valvae simple.

Dilipa morgiana (Westwood)

Common name: The Golden Emperor (Figure 2)

Westwood, [1850]; Gen. diurn. Lep. (2): 305 nota (*Apatura*).

Adult: Fore wing upper side with ground colour dark brown, an oblique light orange coloured band extends from costal margin to termen, another oblique orange coloured post discal band extends from costa to termen, one small and another minute white spot near apex, underside maculation similar as above but quite faded; hind wing basal half of wing dark brown, discal area orange, marginal area very broad and dissected by a zigzag orange coloured streak, under side colour light yellow, no dark markings.

Venation: Fore wing with discal cell shorter than half length of wing, vein Sc slightly stout at base, long and terminates at half costa, R_1 from slightly before end cell, vein R_2 just from upper apex of end cell, stalk of vein R_3+R_4+R_5 present, R_3 ending at apex, M_1 from just below upper apex of end cell, M_2 closer to M_1 than M_3, M_3 just from lower apex of end cell, origin of Cu_1 well before end cell, discal cell closed; hind wing with a bifurcated humeral vein, Sc+R_1 run parallel to costa terminating at wing apex, Sc+R_1 run parallel to costa and ending just below apex, stalk of R_3+M_1+M_2 present, vein M_3, Cu_1 and Cu_2 also stalked, discal cell open.

Male genitalia: Tegumen broad, slightly extended backwards, U-shaped from dorsal view; uncus shorter than tegumen, slender, beak like, tip pointed and curved downward; saccus extremely long, curved upwards at base, tubular, slender, tip swollen, blunt; vinculum narrow, u-shaped from ventral view, longer than latero-ventral projections of tegumen; appendices angulares broad but not curved; valvae simple, well sclerotized, densely setosed with long and fine setae; costa flat, sacculus narrow; ampulla and harpe indistinguishable; apex of valve curved into a pointed hook like tip; aedeagus very long, sinous, well sclerotized, vesica dorsal and membranous; ductus ejaculatorius enters dorsad. .

Female genitalia: Not examined.

Material examined: 1♂, 29.iii.2015, Totu village, Shimla (H.P.)

Distribution: India (Jammu and Kashmir to Arunachal Pradesh, Northeast), Nepal, Bhutan, Myanmar.

Remarks: Genus *Dilipa* Moore was erected on the basis of the type species *Apatura morgiana* Westwood. This genus has only two species, namely, *D. fenestra* (Leech) and *D. morgiana* Westwood. Only the latter species is found in Western Himalaya in India. The nominate species is very rare and has a patchy distribution. It is also included in the Schedule –I of Wildlife (Protection) Act (1972). There are three broods of this species in Western Himalaya and are fond of ripe fruits. The external male genitalia of the nominate species is described and illustrated in detail in the present work.

Genus *Hestina* Westwood

Westwood, [1850]; Gen. diurn. Lep. (2): 281 (35).

*Diagora*Snellen, 1894; Tijdschr.Ent. 37: 67.

*Parhestina*Moore, [1896]; Lepidoptera Indica 3 (26): 34.

*Hestinalis*Bryk, 1938; in Stichel, Lep. Cat. 86: 291.

Type species: *Papilio assimilis*Linnaeus

Linnaeus, 1758; Syst. Nat. (Edn 10) 1: 479.

Body robust; head hairy; eyes glabrous; labial palpi divergent, porrect; antennae equals to half costa, club well defined, long but gradual; thorax robust; discal cell of both wings open.

Remarks: The nominate genus was erected on the basis of type-species *Papilio assimilis* Linnaeus. Only two species, namely *persimilis* (Westwood) and *nicevillei* Moore are found in India. Within India, the former species is distributed from western to eastern Himalaya, whereas the latter is only restricted in western Himalaya. Both the species are rare to find.

Hestina persimilis Butler

Common name: The Siren (Figure 3)

Westwood, [1850]); Gen. diurn. Lep. (2): 281.

Adult: Fore wing upper side ground colour blackish, discal cell with a small white streak and a sinuate spot, end cell covered with three white spots, discal area with broad but scattered white spots, sub-marginal area with a series of white spots, underside maculation as above but upper half of wing lightly coloured than lower half; hind wing upper side from basal area to discal area covered with white maculation, a sub-marginal series of white spots present.

Venation: Forewing with discal cell shorter than half length of wing, vein Sc short and

terminates before half costa, R_1 parallel to Sc and originates well before upper apex of end cell, vein R_2+R_3+R_4+R_5 stalked, R_3 from well before half of vein R_5 and terminates at apex, M_1 just from upper apex of end cell, M_2 closer to M_1 than M_3, M_3 and Cu_1 just from lower apex of end cell, discal cell open, hind wing with a forwardly curved humeral vein, Sc+R_1 parallel to costa margin and terminates at apex, Rs+M_1+M_2 stalked, discal cell open, vein M_3+Cu_1+Cu_2 stalked.

Male genitalia: not examined.

Female genitalia: Ductus seminalis enters dorsally directly into corpus bursae; ductus bursae long, narrow, slender and heavily sclerotized, broad at base, gradually narrow towards corpus bursae, inception at corpus bursae well-marked; corpus bursae round, signum boomerang shaped, well-marked with short spines; apophyses anteriores absent; apophyses posteriores not long, thin, slender, straight with pointed arrow-like apices; papilla analis oval, with proximal margin more sclerotized in middle, pilose.

Material examined: 1♀, 18.v.1971, Shimla (H.P.); 1♀, 26.ix.2015, Andretta, Kangra (H.P.)

Distribution: India (Himachal Pradesh to Arunachal Pradesh, Northeast), Nepal, Bhutan.

Larval host plants: Ulmaceae (Smetacek, 2012).

Remarks: The nominate species is rare and localized in western Himalaya. In India, there are two subspecies *i.e. Hestina persimilis zella* Butler and *Hestina persimilis persimilis* Westwood, found in western Himalaya and eastern Himalaya, respectively. This species can be found frequenting around over ripe fruits. The altitudinal range of this species lies between 1200 m – 2100 m asl. D'Abrera (1985) mentioned its distribution from Nepal and Sikkim which is erroneous, as this species can be recorded from Shimla to Kumaon. The species under reference wonderfully mimics the danaid species *Tirumala limniace* (Cramer) in looks; however, its flight is quite weak as compared to that of the latter.

The external female genitalia of the nominate species has been described and illustrated for the first time in the present manuscript.

Genus *Hestinalis* Bryk

Bryk, 1938; in Stichel, *Lep. Cat.* 86: 291.

*Hestina*Westwood, [1850]; Gen. diurn. Lep. (2): 281 (35).

Type species: *Hestina mimetica* Butler

Butler, 1874; Trans. ent. Soc. Lond. 1874 (4): 426.

Remarks: This genus is mainly centred in south-east Asia. However, only a single species namely *Hestina nama* (Doubleday) is distributed throughout western and eastern Himalaya, India. The species under this genus are admirable mimics of butterflies under genera *Parantica* Moore and *Papilio* Linnaeus.

***Hestinalis nama* (Doubleday)**

Common name: The Circe (Figure 4)

nama Doubleday, 1844; List. lepid. Ins. Brit. Mus. 1: 97 (*Diadema*).

Adult: Fore wing with ground colour deep brown with violet tint, maculation light blue in colour, discal cell with a narrow streak and a rectangular spot, long extended bands in between spaces of veins, two parallel series of irregularly shaped light blue spots in sub-marginal area and marginal area, under side similar as above; hind wing upper side basal colour dark brown, maculation light blue interrupted by dark coloured veins, sub-marginal area very broad with a series of round spots and faded lunular patches in marginal area, underside similar as above, but ground colour bright brown and maculation more prominent.

Venation: Fore wing discal cell shorter than half length of wing, vein Sc long, stout at base and terminates slightly before half costa, R_1 parallel to Sc and from well before upper apex of end cell, stalk of R_2+R_3+R_4+R_5 just from upper apex of end cell, R_2 just from base of stalk and parallel to

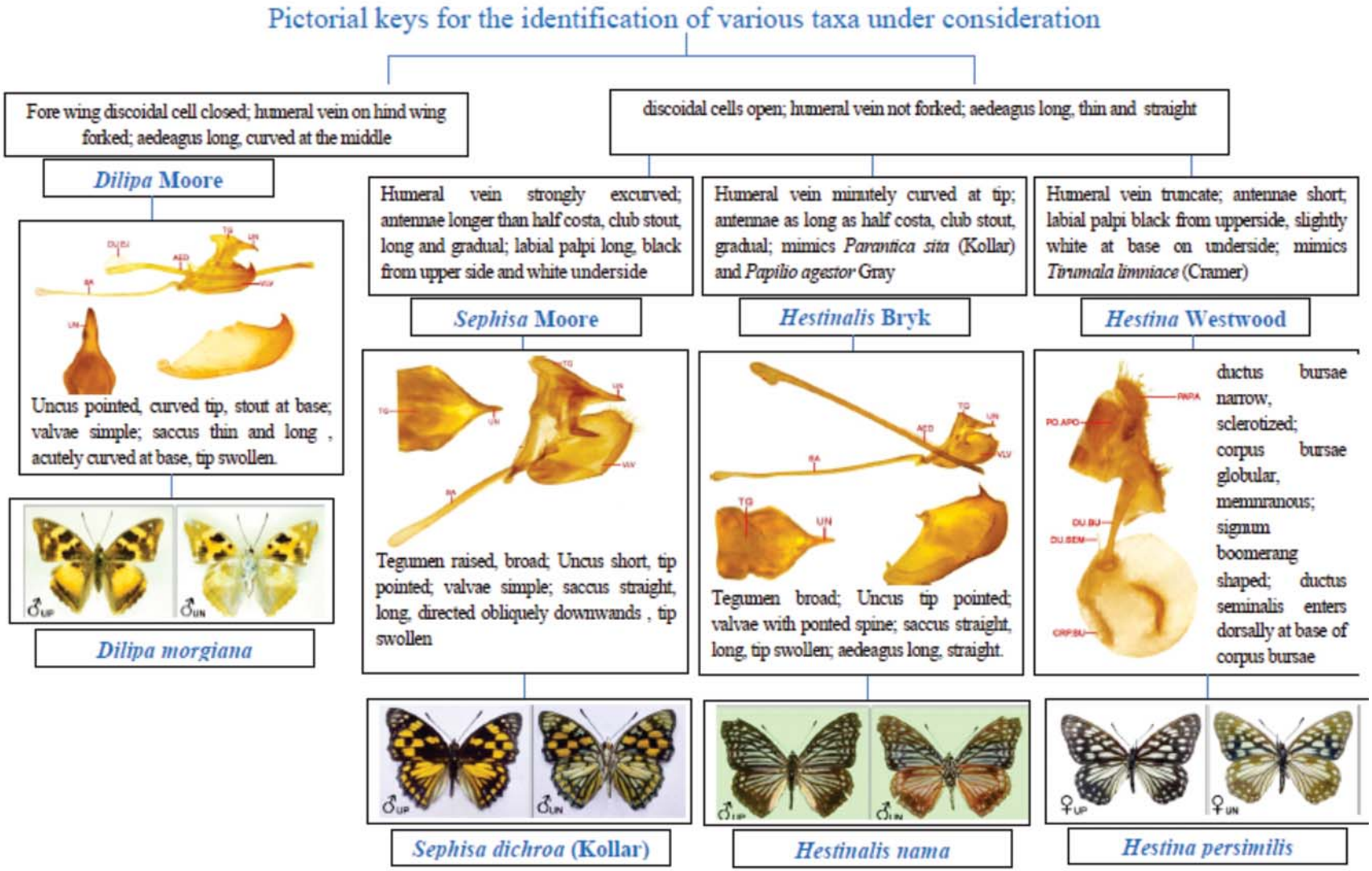

Figure 1: Pictorial keys for the identification of various taxa under consideration.

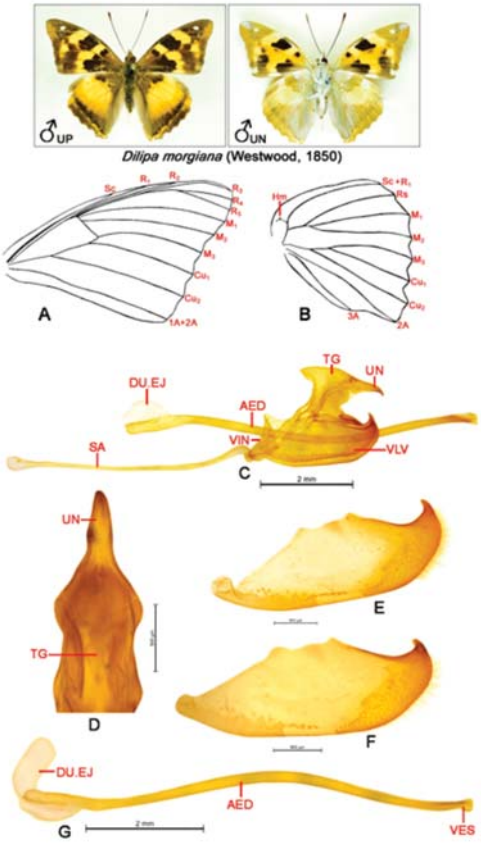

Figure 2: ***Dilipa morgiana*** **(Westwood, 1850); A. Forewing, B. Hindwing, C. Male genitalia, D. Uncus (Dorsal View), E. Valva (Inner View), F. Valva (Outer View), G. Aedeagus.**

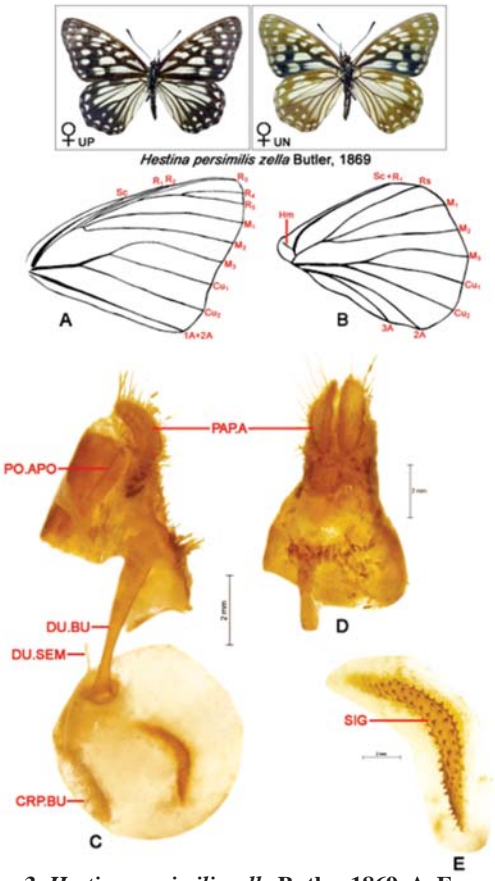

Figure 3: ***Hestina persimilis zella*** **Butler, 1869; A. Forewing, B. Hindwing, C. Female genitalia, D. Papilla analis, E. Signum**

R_1, R_2 and R_3 end on costal margin, R_4 terminates at slightly below apex of wing, M_1 from slightly below upper apex of end cell, M_2 closer to M_1 at origin than to M_3, M_3 deeply, discal cell open, udc and mdc present, ldc absent, hind wing with forwardly curved humeral vein, $Sc+R_1$ run parallel to costa and terminates just at wing apex, stalk of $Rs+M_1+M_2$ and stalk of $M_3+Cu_1+Cu_2$ present, discal cell open, ldc absent.

Adult (Female): not examined.

Male genitalia: Tegumen broad, U-shaped from dorsal view; uncus quite short in length than tegumen, well sclerotized, straight, slender, tip pointed and acutely curved downwards; gnathos narrow but sclerotized, C-shaped from lateral view; saccus very long, slightly curved upwards at base, tip swollen, blunt, slightly sinous; vinculum quite narrow, U-shaped from ventral view; appendix angularea indistinct; valvae simple, well sclerotized, quite broad and somewhat oval n shape, densely setosed with fine and long setae, a sharp spine like extension on dorsal margin of valvae; aedeagus very long, slightly curved, slender, well sclerotized; vesica membranous.

Female genitalia: not examined.

Material examined: 1♂, 5.v. 2015, Andretta, Kangra (H.P.); 1♂, 14.v.2015, Mussoorie, Dheradun (Uttrakhand).

Distribution: India (Himachal Pradesh to Arunachal Pradesh, Northeast), Nepal, Bhutan, Myanmar.

Larval host plants: Urticaceae (Smetacek, 2012).

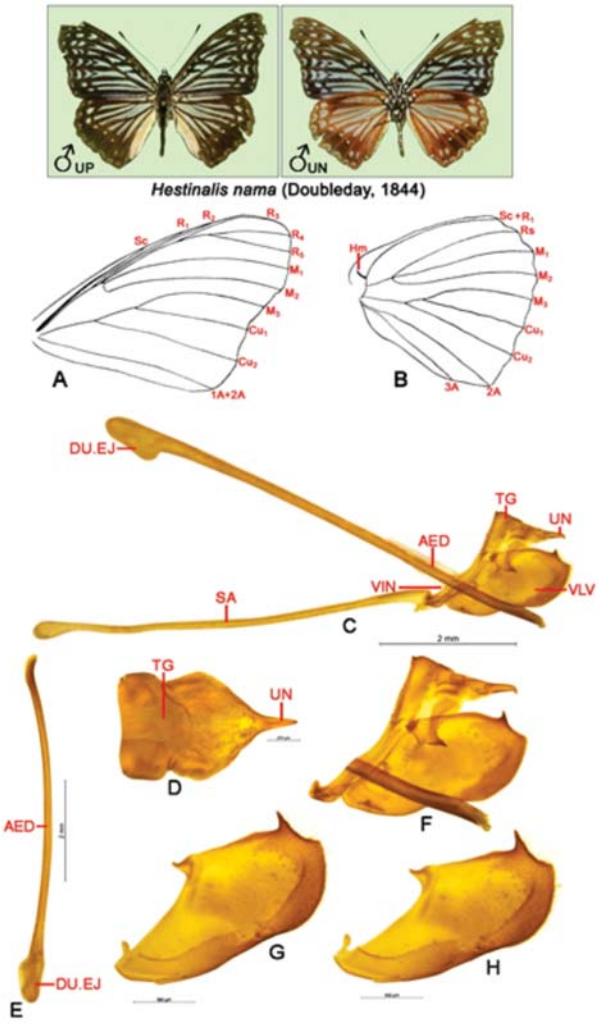

Figure 4: ***Hestinalis nama*** **(Doubleday, 1844); A. Forewing, B. Hindwing, C. Male genitalia, D. Uncus (Dorsal View), E. Aedeagus, F. Male genitalia (Enlarged), G. Valva (Inner View), H. Valva (Outer View).**

Remarks: The taxonomic placement of this species had been problematic in the past. Originally it was described as *Diadema nama* Doubleday. Various other workers designated it as a type species of genus *Hestina* Westwood. However, its recent placement under the genus *Hestinalis* Bryk has been accepted by most workers. The species under reference preferably inhabits the subtropical evergreen forest above 1200m and tropical deciduous forest between 400 and 1400 m asl in Western Himalaya. It is rather a rare species and is known to mimic a danaid species *Parantica sita* (Kollar) and papilionid species *Papilio agestor* Gray, both in looks as well as behaviour. The external male genitalia of the nominate species is described and illustrated in detail in the present work.

Genus *Sephisa Moore*

Common name: The Courtiers

Moore, 1882; Proc. zool. Soc. Lond. 1882 (1): 240.

Castalia Westwood, [1850]; Gen. diurn. Lep. (2): 303.

Castalia Moore, 1857; in Horsfield& Moore, Cat. lep. Ins. Mus. East India Coy 1: 199.

Type species: *Limenitis dichroa* Kollar

Kollar, [1844]; in Hügel, Kaschmir und das Reich der Siek 4: 429.

Body moderately stout; head hairy; eyes reddish and glabrous; antennae longer than half costa, club well defined, gradual and long; labial palpi porrect, oriented obliquely; male genitalia with a very long saccus; female genitalia with sclerotized ductus bursae.

Sephisa dichroa (Kollar)

Common name: The Western Courtier (Figure 5)

S. dichroa Kollar, [1844]; in Hügel, Kaschmir und das Reich der Siek 4: 429 (*Limenitis*).

Adult: Fore wing upper side ground colour blackish brown, discal cell with a dark yellow spot, discal area with prominent dark yellow spots, two light coloured spot near apex, under side maculation similar as above but a white patch at base of wing, three white spots at end cell and apical area with broad but lightly coloured patch ; hind wing upper side with ground colour similar as that of fore wing, discal area occupied with dark yellow maculation, a prominent dark yellow colour spots in sub-marginal area.

Venation: Forewing with discal cell shorter than half length of wing, vein Sc moderately long and terminates on half costa, R_1 parallel to Sc, originate well before upper apex of end cell, stalk of $R_2+R_3+R_4+R_5$ just from upper apex of end cell, vein R_3 well before mid of vein R_5, R_3 terminates

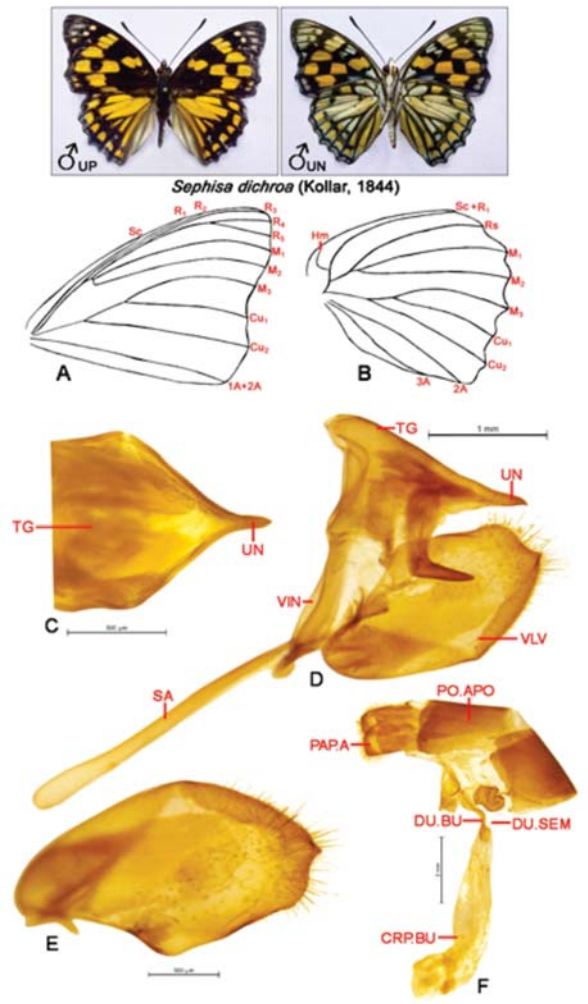

Figure 5: ***Sephisa dichroa*** **(Kollar, 1844); A. Forewing, B. Hindwing, C. Uncus (Dorsal View), D. Male genitalia, E. Valva, F. Female genitalia.**

just at apex of wing, M_1 arises from just below upper apex of end cell, M_2 closer to M_1 at origin than to M_3, M_3 curved, Cu_2 arises just opposite to R_1, hind wing with forwardly curved humeral vein, $Sc+R_1$ parallel to costa and terminates just at apex of wing, stalk of $Rs+M_1+M_2$ and stalk of $M_3+Cu_1+Cu_2$ present, discal cell open, ldc absent.

Male genitalia: Tegumen broad and square in lateral view, slanting towards uncus, well sclerotized, U-shaped from dorsal view; uncus shorter than tegumen, quite broad at base from dorsal view and gradually tapers into a short tubular portion, tip pointed; gnathos well sclerotized, L-shaped from lateral view; saccus quite long, obliquely directed downwards, slender, tip slightly swollen and blunt; vinculum narrow along entire length, approximately as long as latero-ventral projections of tegumen; appendices angulares small, curved and well sclerotized; juxta short, u-shaped, slit like; valvae simple, broad, slightly protruding beyond tip of uncus, densely setose with ling setae.

Female genitalia: Sterigma poorly well developed, lamella antevaginalis reduced to form emarginated sclerotization around ostium bursae, lamella postvaginalis reduced and lightly sclerotized; ductus bursae moderate in length, well sclerotized, slightly twisted; ductus seminalis enters on dorsal side at distal end of ductus bursae; corpus bursae long, elongated balloon like, membranous, tip blunt and swollen, signum absent; apophyses anteriores broad at base, inconspicous; apophyses posteriores moderate, broad at base, tapers to narrow apices; papilla analis rectangular, well sclerotized, distal portion heavily sclerotized, pilose.

Material examined: 1♂, 29.v.2013, Shimla (H.P.); 1♀, 26.ix.2015, Andretta, Kangra (H.P.); 1♂, 27.ix.2015, Barot, Kangra (H.P.).

Distribution: India (Jammu and Kashmir to Uttrakhand), Pakistan, Nepal.

Larval host plants: Fagaceae (Smetacek, 2012).

Remarks: The nominate genus was erected on the basis of the type- species *Limenitis dichroa* Kollar. Only two species are found in India, namely, *Sephisa chandra* (Moore) and *Sephisa dichroa* Kollar. The former is distributed in the eastern parts of India, whereas, the latter in western Himalaya. It is a common species and is fond of sucking sap from ripe fruits, and can be occasionally found around human inhabited places. The external male and female genitalia of the nominate species has been described and illustrated for the first time in the present work and these characters have been used for upgrading of genus diagnosis.

Discussion: Four species referable to four genera under the subfamily Apaturinae were taxonomically studied under the present work. The morphological

characters of external male and female genitalia for the species under reference have been studied for the first time. However, the number of specimens studied was limited to only seven because of the rarity of the species. The species *Dilipa morigana* (Westwood) is protected under Schedule-I of Wildlife (Protection) Act (1972); *Hestina persimilis* Butler is rare; *Hestinalis nama* (Doubleday) and *Sephisa dichroa* (Kollar) are uncommon in the western Himalaya. One of the shortcomings of the present study is that intra-population variations could not be studied due to small sample size. Moreover the male of the *H. persimilis* Butler and female of the *D. morigana* (Westwood) and *Hestinalis nama* (Doubleday) could not be collected. The future studies on this group should be aimed at filling these gaps. Moreover, regular surveys should be conducted to update the population status of these species.

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ABBREVIATIONS used

♀UN	Female Under side	M_3	Third median vein
♀UP	Female Upper side	mdc	Middle disco-cellular vein
♂UN	Male Under side	PAP.A	Papilla analis
♂UP	Male Upper side	PO.APO	Posterior apophyses
1A	First anal vein	R_1	First radial vein
2A	Second anal vein	R_2	Second radial vein
AED	Aedeagus	R_3	Third radial vein
AMP	Ampulla	R_4	Fourth radial vein
ANT.APO	Anterior apophyses	R_5	Fifth radial vein
CO	Costa	Rs	Radial sector
CRN	Cornuti	SA	Saccus
CRP.BU	Corpus bursae	$Sc + R_1$	Stalk of SC and R1
Cu_1	First cubital vein	Sc	Subcosta
Cu_2	Second cubital vein	SIG	Signum
DU.BU	Ductus bursae	SL	Sacculus
DU.EJ	Ductus ejaculatorius	TG	Tegumen
HRP +AMP	Harpe and Ampulla (fused)	udc	Upper disco-cellular vein
JX	Juxta	UN	Uncus
ldc	Lower disco-cellular vein	VES	Vesica
M_1	First median vein	VIN	Vinculum
M_2	Second median vein	VLV	Valva

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Diversity of coccinellid beetles (Coccinellidae: Coleoptera) in Kashmir, India

Razia Rasheed* **and A. A. Buhroo**

Entomology Research Unit, Post Graduate Department of Zoology, University of Kashmir, Srinagar 190006, Jammu & Kashmir, India. Email: raziarasheed123@gmail.com

ABSTRACT: Surveys were conducted on the diversity of coccinellid beetles in the horticultural ecosystems namely fruit orchards, vegetables and wild vegetation ecosystem of Kashmir during June 2014 to June 2015. Diversity indices like Shannon Weiener index; Simpson index; Margalef's index and Pielou index were used for studying diversity and abundance of coccinellid beetles. The results revealed that 1536 specimens of ladybird beetles collected, were identified into three sub families, 11 genera and 13 species. The diversity indices showed good diversity and rich fauna of coccinellids. The study brought the fact that the coccinellids are evenly distributed throughout the study area. Comparison of abundance, species richness and diversity indices among fruit, vegetable and wild vegetation ecosystems revealed that coccinellid beetle diversity was more in wild vegetation and fruit ecosystems due to availability of prey as compared to vegetable ecosystem.

KEY WORDS: Diversity indices, Shannon Weiener index, Simpson index, Margalef's index, Pielou index, coccinellid beetles, agro-ecosystem, Kashmir

INTRODUCTION

Insects represent a dominant component of biodiversity in most terrestrial ecosystems and play a significant role in the ecosystem functioning (Weisser and Siemann, 2004). Loss of biodiversity is one of the major causes leading to environmental degradation. With the increase in population, there is more demand for food and it shows the importance of agricultural intensification. To improve the crop yield by using fertilizers and pesticides resulted in contamination and disturbance in natural ecosystems, ultimately harming biodiversity and community health (Hughes *et al*., 2002). Predation may increase the biodiversity of communities by preventing a single species from becoming dominant. It is obvious that predators depend on prey for survival, and this is reflected in predator populations being affected by changes in prey populations. Predators may be put to use in conservation efforts to control introduced species. Besides their use in conservation biology, predators are also important for controlling pests in agriculture. Natural predators are an environmental friendly and sustainable way of reducing damage to crops, and are one alternative to the use of chemical agents such as pesticides (Stanley, 2008).

Among predatory insects, coccinellids are one of the most economically important groups and are very widespread in agriculture and forest ecosystems. They solely feed on a number of

* Author for correspondence

distantly related phytophagous insect pests of the agriculture and horticultural crops (Hodek and Honek, 1996; Omkar and Parvez, 2000). Coccinellids are also regarded as bio indicators (Iperti and Paoletti, 1999) and provide more general information about the ecosystem in which they occur (Anderson, 1999). They play their important role as bio control for those crops that are especially susceptible to aphid attack, namely maize, apple, vegetables, pear etc. Not only aphids, scales are also destructive pests of fruit orchards reducing fruit quality and quantity; these predators can significantly contribute in controlling these pests (Mulvany, 2002). Therefore, objective of the present study was to examine the species composition and diversity indices of coccinellid beetles in south Kashmir.

MATERIALS AND METHODS

Three districts were selected from south Kashmir, India viz., district Anantnag (33° 43´ N and 75° 09´ E), Pulwama (33° 98´ N and 75° 01´ E) and Shopian (33° 71´ N and 74° 83´ E) (Fig.1). Survey was conducted from June 2014 to June 2015. Collection was done from horticultural ecosystems namely fruit orchards, vegetables and wild vegetation ecosystem of these regions.

Sampling methods: Sampling was conducted in different horticulture ecosystems of study area. Sampling was carried out from first week of June 2014 till late June 2015. Beetles were collected by net sweeping method and hand picking method (Jonathan, 1995). The net used for collection was made of white muslin cloth with long handle. Hand picking method was mostly adopted for collection. Sampling was done at fortnightly interval. Random sampling was done by choosing 10 fruit trees from each fruit orchard and 10 quadrants (1 square meter each) from crop land ecosystem from each location. The collected specimens were kept in collecting jars and collection tubes and brought to Entomology laboratory Department of Zoology, University of Kashmir for identification.

Identification: The collected specimens were identified with the help of available literature and taxonomic keys. The keys consulted during present study include Kapur (1956, 1958, 1963 and 1967) and Kuznetsov (1997).

Calculation of diversity indices: To calculate the diversity of ladybird beetles, following indices were used.

Shannon-Weiener index (Shannon, 1948).

$$H = -\sum_{i=1}^{s} (P_i \log_e P_i)$$

Where,

H = Shannon Weiener index

P_i = proportion of "ith" species and is calculated as "ni/N", where, "ni" is the number of individuals in "ith" species and N is the total number of individuals in the sample.

$\log_e p_i$ = Natural log of P_i

Simpson's index (D) (Simpson, 1949):

$$D = \text{sum } (P_i^2)$$

Simpson's reciprocal diversity index = 1/D

Where,

P_i = proportion of "ith" species and is calculated as "ni/N", where, "ni" is the number of individuals in "ith" species and N is the total number of individuals in the sample.

Margalef's index (M_a) (Margalef, 1968, 1969) / Species Richness (Pielou, 1975).

$$M_a = S\text{-}1/\log_e N$$

Where,

S = number of species; N = total number of individuals; $\log_e$ = natural log

Species Evenness index (E) or (J) (Pielou, 1969)

$$E = H/\log_e S$$

Where,

H = Shannon - Weiener index; S = number of species; $\log_e$ = natural log

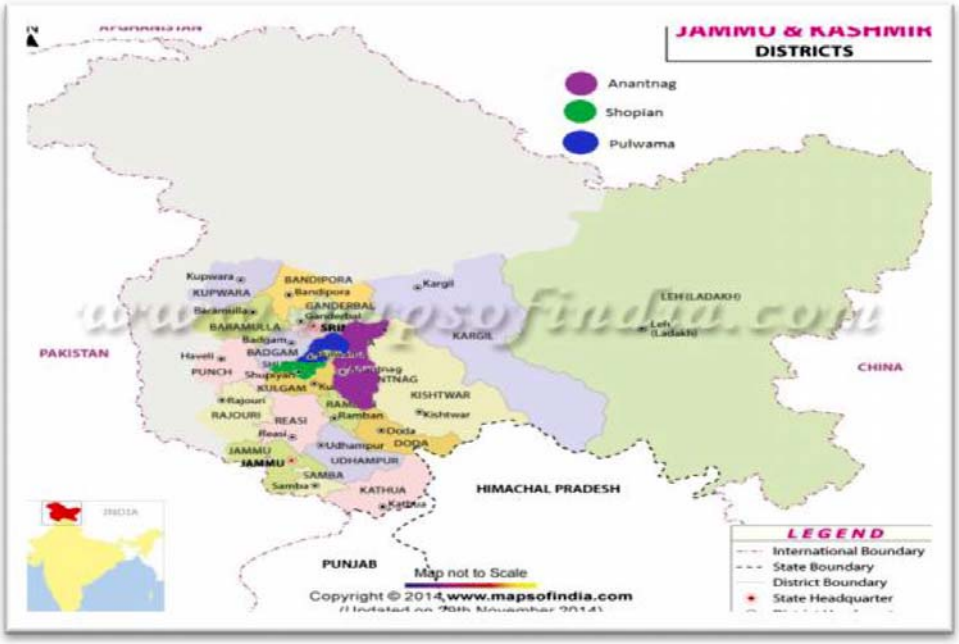

Fig.1. Map showing study sites

RESULTS

During the present study 1536 specimens of ladybird beetles were collected from study sites, which were identified into 3 sub families, 11 genera and 13 species. Total number of specimens collected from district Anantnag was 555, from district Pulwama 522 and from district Shopian 459 (Table 1).

The calculated values of Shannon - Weiener index at different districts ranged from 2.33 (Anantnag) to 2.29 (Shopian). The lowest diversity index was

Table 1. Total number of specimens collected from three districts of South Kashmir

SPECIES	Total number of specimens collected			TOTAL
	ANANTNAG	PULWAMA	SHOPIAN	
Coccinella septempunctata	122	97	89	308
Chilocorus infernalis	105	99	90	294
Adalia tetraspilota	87	76	81	244
Hippodamia variegata	41	68	35	144
Oenopia conglobata	15	21	27	63
Coccinella transversalis	37	18	31	86
Coccinella undecimpunctata	30	15	39	84
Harmonia dimidiata	24	36	22	82
Macroilleis hauseri	26	19	09	54
Calvia punctata	25	41	12	78
Illeis indica	09	06	04	19
Henosepilachna vigintioctopunctata	16	14	12	42
Platynaspidius saundersi	18	12	08	38
Total = 13	555	522	459	1536

calculated from district Shopian (2.29) and district Pulwama (2.30). The highest value was from district Anantnag (2.33) (Table 2). The data computed by the Shannon Wiener index revealed that coccinellid beetles are more or less equally distributed at all districts because the calculated values did not show much difference among the three districts. Similarly, the calculated values of Simpson index ranged from 0.131(Shopian) to 0.093 (Pulwama). The lowest Simpson index was calculated from district Pulwama (0.093) and district Anantnag (0.129) whereas highest value was calculated from district Shopian (0.131). This index showed that lowest abundance was obtained from district Pulwama and Anantnag and highest abundance was obtained from Shopian. All the values obtained from this index showed that coccinellid beetles abundance is more or less same for all the districts surveyed during present work. Similarly Simpson's reciprocal diversity index ranged from 10.73 (Anantnag) to 7.63 (Shopian) (Table 2).

The calculated values of Margalef's index ranged from 1.95 (Shopian) to 1.89 (Anantnag). The lowest value was obtained from district Anantnag (1.89) and district Pulwama (1.92) and highest from district Shopian (1.95). This indicates that species richness was slightly higher at Shopian district. Likewise the calculated values for species evenness ranged from 10.73 (Pulwama) to 7.63 (Shopian) (Table 3).

Table 3. Calculated values of Evenness and Richness at three districts

Location	Evenness	Richness
Anantnag	0.910	1.89
Pulwama	0.898	1.92
Shopian	0.895	1.96

Table 2. Calculated values of diversity indices of three districts

Study sites	Shannon Wiener Index	Simpson Index	Simpson Reciprocal Index	Simpson Index of Diversity
Anantnag	2.33	0.129	7.75	0.871
Pulwama	2.30	0.093	10.73	0.906
Shopian	2.29	0.131	7.63	0.868

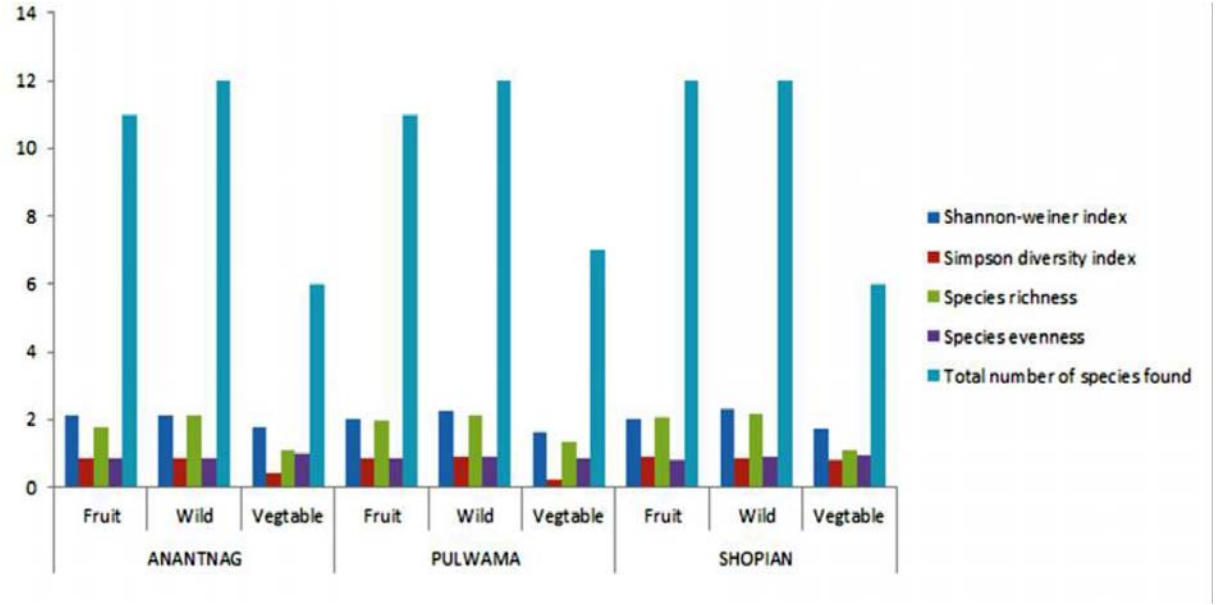

Fig. 2. Graph showing diversity indices among three ecosystems

Table 4. Diversity indices of ladybird beetles in fruit, vegetable and wild vegetation ecosystems at three sites

Diversity indices	ANANTNAG			PULWAMA			SHOPIAN		
	Fruit	Wild	Vegetable	Fruit	Wild	Vegetable	Fruit	Wild	Vegetable
Shannon - Weiener index	2.11	2.12	1.76	2.02	2.25	1.62	2.01	2.29	1.72
Simpson diversity index	0.84	0.85	0.42	0.83	0.88	0.22	0.88	0.86	0.81
Species richness	1.78	2.12	1.09	1.98	2.13	1.34	2.05	2.16	1.11
Species evenness	0.87	0.87	0.98	0.84	0.9	0.83	0.81	0.92	0.96
Total species (no.)	11	12	6	11	12	7	12	12	6

Different diversity indices were also applied to three different ecosystems in each district for calculating the diversity of coccinellid beetles in particular ecosystem. The three ecosystems include fruit, vegetable and wild vegetation ecosystems. The calculated values of different indices showed that fruit ecosystem and wild vegetation have diverse assemblage of coccinellids as compared to vegetable ecosystem (Fig.2). They were also found to support higher number of coccinellid species. In all, 11 species of coccinellids were found in fruit ecosystem of district Anantnag and Pulwama while as in Shopian 12 species were found. Likewise in Wild vegetation 12 species were found from all the three districts. In Shopian district and Anantnag six species were found from Vegetable ecosystem and seven species in district Pulwama (Table 4).

Also during the present study, the most encountered species was *Coccinella septempunctata.* It was found dominating species from all the three districts and abundantly present in all the three ecosystems. Very interestingly *Henosepilachna vigintioctopunctata* showed narrow range of habitat and was collected only on vegetable ecosystem. On the other hand *Chilocorus infernalis* was absent in vegetable ecosystem in all three districts and showed dominance in fruit ecosystem and wild vegetation.

DISCUSSION

The results obtained during present study showed the diversity of coccinellid beetles in horticulture ecosystem in south Kashmir. A total of 13 species were recorded during the survey. Bhagat *et al.* (1988) reported 12 species of coccinellid beetles from apple orchards of Jammu and Kashmir. Azim and Bhat (2005) published the taxonomic notes of eight coccinellid beetles from Kashmir, two species from subfamily Chilocorinae and six from subfamily Coccinellinae. The different diversity indices used during present study was similar to that of indices used by Hayat and Khan (2013) and Biranvand *et al*. (2014). The present results showed rich diversity of ladybird beetles in fruit ecosystem and wild vegetation as compared to vegetable ecosystem. These findings are in accordance with those of Shah and Khan (2014) and Khan *et al*. (2007 a, b).

The study showed great diversity and rich fauna of coccinellid beetles in the South Kashmir recording 13 different species belonging to 11 genera and three subfamilies. The various diversity indices like Shannon - Weiener index, Simpson index; Margalef's index and Pielou index showed that the species recorded during the present study are evenly distributed throughout the study area. Comparison of abundance, species richness and diversity indices among fruit, vegetable and wild vegetation ecosystems revealed that coccinellid diversity was more in wild vegetation and fruit ecosystems due availability of prey as compared to vegetable ecosystem which are of short duration. Thus it can be suggested that fruit and wild vegetation can act as important natural habitats of coccinellid predators as they were found to support higher number of coccinellid beetles. The ability of these coccinellid beetles to be so successful in a

large range of habitats makes it especially beneficial to humans who need crop security from aphid infestations.

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Effect of thermal variation on protein contents in the haemolymph of multivoltine mulberry silkworm *Bombyx mori* Linn.

S.K. Gupta[1] and K.P. Gaur[2]

[1]Department of Zoology, Govt. Girls P G College, Ghazipur 233001 U.P., India;
[2]Silkworm Laboratory DDU Gorakhpur University Gorakhpur 273009 U.P., India.
Email:drsanjaigupta1976@gmail.com

ABSTRACT: Investigation on different developmental stages of *Bombyx mori* revealed that the total protein content of haemolymph was influenced significantly with the variation in temperature. The maximum level (33.81µg/mg) of total protein content was notice in the haemolymph of fifth instar larvae, reared at 26°C while the minimum level (10.86µg/mg) was recorded in the adults reared at 14°C.

KEY WORDS: *Bombyx mori*, temperature, silkworm, haemolymph protein

INTRODUCTION

The study on the effect of temperature may provide good understanding of various life processes; therefore a possible ideal ecological model with particular reference to temperature may be formulated for the success of sericulture industry. Being poikilotherm the body temperature of *Bombyx mori* Linn. is variable in accordance with the environmental temperature influencing the developmental process, silk producing potential and biochemical constituent (Mortimer *et al*., 2013).The secretion of silk is a complex process which involves a chain of enzymatic and biochemical process as a result the level of various chemical constituent like protein, free amino acid and nucleic acids may also be influenced due to temperature variation. It is well know that temperature plays a major role in their physiological and biochemical behaviour of the insect. The insects will get acclimatized to low temperature by the production of various cryoprotectants like glycerol, trahalose and sorbitol (Sinclair, 2003). There is a direct apart from the above studies it is also reported that correlation among the number of cells size of gland cells and the amount of silk production by amino acids and haemolymph (Shimizu and Horiuchi, 1952). Keeping these views an attempt has been made to investigate the effect of varying temperature on the protein contents in the haemolymph of Nistari race of *B. mori* which affect the rearing and cocoons parameters.

The seed cocoons of multivoltine mulberry silkworm were obtained from the silkworm grainage Bahraich, Directorate of Sericulture, Uttar Pradesh and were maintained in plywood trays (23×20×5cm) under the ideal rearing condition in the laboratory. The

* Author for correspondence

temperature and RH were maintained at 26±1°C and 80±5% respectively till the emergence of moths from the seed cocoons.

The whole grainage operation was performed as per description given by (Krishnaswami *et al*., 1973). To observe the effect of temperature on the performance of *B. mori* larvae, an experiment was performed at different temperature regimes like 10, 14, 18, 26, 34, and 38°C. At 38°C larvae did not survive after the fourth instar stage. The experiments were conducted in BOD incubator separately one after another. The optimum condition of the experiments like 26±1°C temperature, 80±5% RH and 12 hrs light a day were taken as control for all the experimental designing were similar to Gaur and Upadhyay (2001). After four hours of mating moths were decoupled manually and transferred chronically to BOD incubator maintained 10°C (one of the six experimental temperature regimes), 80±1% RH and 12 hrs light a day. The egg laying moths were covered by open plastic cellules to prevent the intermixing of egg masses deposited by different female moths after 24 hrs of egg laying, the female moths were individually examined for their diseases freeness.

The disease free laying (DFLs) were washed with 2% formalin for 15 minutes to increase the adhesiveness of egg over card on the surface .Thereafter the egg sheets with egg laid on were thoroughly washed with running water to remove the formalin and the egg were dried in shade and transferred chronically transferred to specific experimental condition for further rearing.

To observe the effect of temperature variation on the protein contents present in the haemolymph of experimental *B. mori* were dissected at day 3rdof 4^{th}, 5^{th} instar larvae pupae and adult stage haemolymph was taken out. In the present study the estimation of protein formed in the haemolymph was made according to the method of Lowery *et al*. (1951) as modified by Singh and Agrawal (1989). The values of protein content in haemolymph were analysed statistically by two way ANOVA.

The data clearly demonstrate the changes in the level of the total protein content in the haemolymph of *B. mori* during different developmental stages. The total protein content in the haemolymph of fourth instar larvae was considerably influenced by the variation in rearing temperature regimes. With the gradual increase in temperature from 10 to 26°C, the total protein content increased from 16.40µg/mg at 10°C, to the maximum level of 28.74µg/mg at 26°C while further increase in temperature from 26 to 38°C caused gradual decrease in the total protein content which reached to the lower level of 21.55µg/mg at 38°C. Similarly the protein content in the haemolymph of fifth instar larvae was influenced considerably due to variation in rearing temperature. With the increasing temperature from 10 to 26°C, the total protein content increased from 16.67µg/mg at 10°C, to the maximum level of 33.81µg/mg at°C. But further increase in temperature above 26°C caused gradual decline in the total protein content, which reached to the lower level of 23.64µg/mg at 34°C. At 38°C larvae did not survive after fourth instar stage. The total protein content in the haemolymph of pupae was also influenced due to the variation in rearing temperature. With the variation in temperature from 14 to 26°C the total protein content increased from 12.46µg/mg at 14°C to the maximum level 18.01µg/mg at 26°C while further increase in the temperature from 26 to 34°C caused gradual decreased in the total protein content which reached to the level of 16.62 µg/mg at 34°C. At 10° and 38°C larvae were unable to pupate. Further the protein content in the haemolymph of adult was influenced due to varying temperature regimes. With the increasing temperature from 14 to26°C the total protein content increased considerably from 10.86µg/mg at 14°C to the maximum level of 16.76µg/mg at 26°C. At 10° and 38°C adults were unable to emerge. The total protein content in the haemolymph with the varying temperaturewas almost found to be of increasing trend from 10° to 26°C while decreased above 26°C. The maximum level 33.81µg/mg of total protein was recorded in the haemolymph, obtained from the fifth instar larvae, reared at 26°C. The minimum level 10.86µg/

Table 1 Effect of temperature on protein content (µg/mg) in the haemolymph of different stages of *Bombyx mori*

Stages	Protein content (µg/ mg) in the haemolymph at — Temperature (^{0}C)						$F_{1.}$ ratio n_1=5
	10	14	18	26	34	38	
IV instar	16.40±0.52	18.63±0.53	21.16±0.58	28.74±0.59	22.61±0.84	21.55±0.96	**5.26***
V instar	16.67±0.51	19.52±0.39	21.36±0.42	33.81±0.65	23.64±0.55	N.Sd	
Pupa	N.Sd	12.46±0.41	14.69±0.52	18.01±0.78	16.72±0.53	N.Sd	
Adult	N.Sd	10.86±0.31	12.92±0.13	16.76±0.34	15.10±0.14	N.Sd	

F_2 ratio = 5.67* n 2- 8 N.Sd= Not survived *P<0.025

Each value represents mean ± S.D of six replicates

mg of protein was recorded in the haemolymph, obtained from the adult reared at 14°C. Two way ANOVA indicates that the variation in the temperature and developmental stages have significant (P<0.025) influenced on the total protein content in the haemolymph of *Bombyx mori* (Table 1).

Silk is made up of two protein such as fibroin and sericin. Fibroin forms the core and is surrounded by sericine. These two differs in their characteristic and secreted from different parts of silk gland. A decreased protein content of haemolymph was recorded in *Rhodnius prolixus* at high temperature regimes (Okasha, 1964). Protein synthesis in the haemolymph was suggested to be stimulated at low temperature 15°C by increased neurosecretory activity in *Lucusta migratoria* (Clarke, 1967). In Drosophila melanogaster, protein concentration in the haemolymph seemed to increase marginally in insects reared at 25°C over those maintained at 15°C (Singh and Dass, 1982). By knowing the economic importance and convenience, silkworm has almost become an important tool for several biochemical physiological and genetic studies in insects. Physiological and biochemical studies includes metabolism an morphogenesis in insect, digestion and digestive enzyme, protein synthesis and their metabolism, hormones and their mechanism of action structure and function of chromosomes etc., for better productivity. Major biomolecules such as carbohydrates lipids protein, hormones and chromosomes etc play an important role in biochemical process underlying growth and development of insects (Ito and Horie, 1959). Metabolism and accumulation of these biomolecules in insect tissues during their development in different stages of life cycle was studied by Tanaka and Kusano (1980), Friedman (1985) and Bhattacharya and Kanwal (2004). The concentrations of these biomolcules mainly depend on mulberry leaf quality.

In different developmental stages of Bombyx mori the total protein content of haemolymph was significantly with the variation in temperature. The maximum level 33.81µg/mg of total protein content was noticed in the haemolymph of fifth instar larvae reared at 26°C while the minimum level 10.86µg/mg of that was recorded in the haemolymph of the adult reared at 14°C. Our investigation especially temperature affects the biochemical changes which affects the cocoon morphology as well as its stiffness and strength which we attribute to altered spinning behaviour and sericine curing time. Biochemical change affects cocoon colouration perhaps due to tanning agents. Finally the haemolymph content of cocoon modifies sericin distributed and stiffness without changing toughness. Our findings demonstrate environmentally induced quality parameters that must not be ignored when analyzing and deploying silk cocoon, silk filaments or silk-derived bio-polymers.

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Natural incidence of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) on okra (*Abelmoschus esculentus* (L.) Moench)

M. Shankara Murthy*, S. K. Nagaraj and A. Prabhuraj[1]

Department of Entomology, College of Agriculture, Bheemarayanagudi 585 287, UAS, Raichur, Karnataka, India. [1]Department of Entomology, University of Agricultural Sciences, Raichur 584 104, Karnataka, India. Email:smurthyent@gmail.com

ABSTRACT: Natural incidence of pink bollworm, *Pectinophora gossypiella* on okra, its nature and extent of damage is reported. Adult external morphology and characters of male and female genitalia are described with photographs. © 2018 Association for Advancement of Entomology

KEY WORDS: India, adult morphology, male and female genetalia, natural infestation

The cotton pink bollworm, *Pectinophora gossypiella* (Saunders) was described by W.W. Saunders in 1843 as *Depressaria gossypiella,* based on specimens found damaging cotton in India. Later, Common (1958) placed the species *gossypiella* in the genus *Pectinophora* in his revision of pink bollworms of cotton and related genera in Australia. It was reported as a serious pest of cotton in German East Africa during 1904 (Vosseler, 1904). Since then, it has become one of the globally distributed noxious pests of cotton. The pest is also reported to cause damage or survive on a broad range of host plants representing seven families, 24 genera and 70 species mostly belonging to the family Malvaceae (Busck, 1917). However, the natural incidence of this pest on okra has not been documented so far from India. Earlier, Butani and Verma (1976) recorded 20 species of insects on okra except *P. gossypiella*. Similarly, Pal *et al.* (2013) studied the incidence of insect pests on okra in West Bengal, and recorded eleven species, excluding *P. gossypiella*. Sharma *et al.* (2008) studied the biodiversity of lepidopteran insects associated with vegetables in India, and recorded 152 species including *P. gossypiella.* Further, Sharma (2011) studied the lepidopteran insects associated with vegetables in Aravali Range of Rajasthan, and recorded 38 species together with *P. gossypiella.* Chakraborty *et al.* (2014) also studied the biodiversity of insect fauna on okra and recorded 112 pest species including *P. gossypiella*. However, none of the above studies confirm feeding or breeding of *P. gossypiella* on okra in India as these reports are based on collection of adults manually, at light or using aspirator or sweep nets. Information on the male and female genital characters of *P. gossypiella* from India is also lacking. However, Busck (1917) described these characters only through line diagrams for the specimens collected on cotton from USA. Here we report the natural incidence of *P. gossypiella* on okra in India, its nature and extent of damage

* Author for correspondence

Plate 1: Natural incidence of *Pectinophora gossypiella* (Saunders) on okra
a) Early instar larva inside the furit, b) Grown-up larvae feeding on seeds, c. Pupa in the damaged fruit

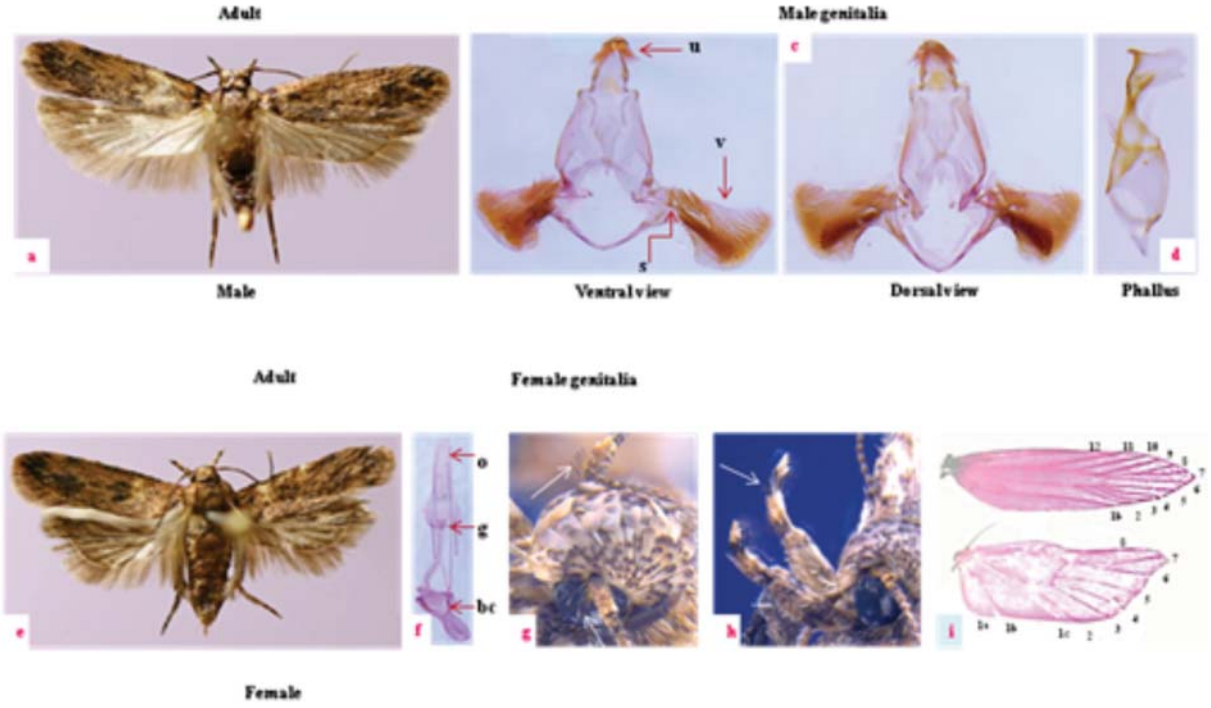

Plate 2: Genital and morphological characters of adult *Pectinophora gossypilla* (Saunders)
a) male; male genetalia, b) ventral view, c) dorsal view, d) phallus, e) female, f) female genitalia, g) basal segment of antennae with stiff, long, hair-like scales; h) labial palpi are long and curved upwards, i) wing venation

for the first time. Adult external morphology and characters of male and female genitalia are described and photographs are provided for easy identification of the pest.

Incidence of *P. gossypiella* on okra was observed during 2016 - 17 in the experimental block of Agriculture College, Bheemarayanagudi (411m, 16°71'N 76°75'E), Karnataka (India). Sampling was done to quantify the extent of damage on okra. Three hundred and eleven fruits were collected randomly from the field, of which seven were infested with *P. gossypiella* (2.25 % damage) (Plate 1, a - c). The neonate larva was pale creamy white initially, later turned to pink as it grew. It was noticed feeding on seeds inside the fruit (Plate 1, a - c).

To study the adult morphological and genital characters, larvae collected from the infested fruits during field survey were reared on the same host to adult stage in the laboratory. The emerged adults were killed, pinned, stretched, labelled, dried properly and preserved in the Department of Entomology, College of Agriculture, Bheemarayanagudi, University of Agricultural Sciences, Raichur 584 104, Karnataka, India. The morphological as well as genital characters of the adults of *P. gossypiella* were studied following Hampson (1896), Clark (1941) and Robinson (1976) with the necessary modifications. Before dissection of genitalia, adult specimens were photographed. Adult structures such as fore and hind wings, palpi and genitalia were photographed using Trinocular microscope with auto-montage (Leica M205C). The identity of the species was confirmed based on Busck (1917).

Description: Adult (Plate 2 a, e, g & h) is a small, dark-brown moth measuring 12-20 mm across the wings. Head red brown with pale, iridescent scales. Antennae brown, basal antennomere with a pecten of five or six long, stiff, hair-like scales. Labial palpi are long, curved upward. Forewings elongated, oval, pointed at tips, bearing a wide fringe of hairs. Hind wings broader than fore wings, trapezoidal, silvery grey with darker, iridescent hind margin. The wing fringe ochreous.

Wing venation: Forewing with 12 veins. Veins 7, 8 stalked. Veins 4, 5 well separated at origin. Vein 3 before angle of cell. 1b furcate at base. Hind wings with 8 veins. Costa deflected from middle, apex pointed. Vein 8 connected with cell by oblique bar. Veins 6, 7 closely approximated at base. Veins 3, 4 connate. Vein 5 parallel with 4 (Plate 2, i).

Male genitalia: Valvae (v) narrow at base, broadening towards tip; tip strongly haired with a cluster of long, heavy, straight spines from its inner side. Sacculus (s) armed on its edge with a row of stout spines. Uncus (u) moderately long, broad at base, tapering towards tip and heavily hairy. Phallus is short, stout with a terminal hook (Plate 2, b-d).

Female genitalia: Ovipositor (o) is weakly chitinized, covered with stiff hairs. Genital plate (g) heart shaped. Bursa copulatrix (bc) with two opposite, strongly chitinized, horn like serrated invaginations (Plate 2, f).

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Comparative biology of *Helopeltis theivora* Waterhouse (Heteroptera: Miridae) on *Psidium cattleianum* (Myrtales: Myrtaceae) *and Camellia sinensis* (Ericales: Theaceae)

Anju Antony, K.K. Srikumar and B. Radhakrishnan*

Department of Entomology, UPASI Tea Research Foundation, Tea Research Institute, Valparai 642 127, Tamil Nadu, India.
Email: anjuantony34@gmail.com; *director@upasitearesearch.org*

ABSTRACT: Studies were carried out on the life history of *Helopeltis theivora* on *Psidium cattleianum* shrub is an alternate host of *H. theivora*. Biology of *H. theivora* on this shrub was studied for the first time. Incubation period varied for 8.6 ± 0.3 (d). Total nymphal developmental period was 20.3 ± 2.84 (d). Adult female lived longer than male. Incubation period and the total nymphal developmental period of *H. theivora* on *Psidium cattleianum* was significantly lower than *Camellia sinensis*.

KEY WORDS: *Helopeltis theivora, Psidium cattleianum*, *Camellia sinensis*, biological parameters

Camellia sinensis L.O. (Kuntze) a perennial crop and grown as a monoculture over large continuous areas during the last 160 years had formed a stable tea ecosystem for widely divergent endemic or introduced pests (*Mamun et al*., 2014). More than one thousand species of arthropod pests are known to attack tea all over the world, though only about 300 species of insects are recorded from India (Das, 1965). Among them, *Helopeltis theivora* (Heteroptera: Miridae) is a major pest of cocoa and tea in India and other countries of Asia. It has also been reported damaging other economically important plants such as black pepper, camphor, cashew and cinchona (Stonedahl, 1991). Typical feeding damage by *Helopeltis* spp. appears as a discoloured necrotic area or a lesion around the point of entry of the labial stylets inside the plant tissue (Srikumar and Bhat, 2012). In a severe attack, bushes virtually cease to form shoots and the affected area may not flush for weeks (Ahmed, 1996). *Psidium cattleianum* Sabine (Myrtaceae), the strawberry guava, was recorded as important alternate host for this pest. *Psidium cattleianum* is an evergreen shrub or small tree to 8 m (25 ft) tall, with gray to reddish-brown peeling bark and young branches round and pubescent. Leaves are opposite, simple, entire, glabrous, elliptic to oblong, to 8 cm (3 in) long (www.fleppc.org/ID_book/psidium/20cattleianum). The present investigation was conducted to study the male and female developmental rate, incubation period, nymphal, adult longevity and fecundity of *H. theivora* on *P. cattleianum* and *C. sinensis*

Life history of *H. theivora* was studied under laboratory conditions at UPASI Tea Research Foundation Valparai, India. Adults (No. 20) of *H. theivora* were collected from UPASI tea fields by using long test tubes (17.5 cm × 3 cm). Adult bugs were allowed to pair on different bottles fed with

* Author for correspondence

Table 1. Life history of *Helopeltis theivora* on *P. cattleianum* and *C. sinensis*

Host	Nymphal development period (in days)					Total development period (in days)		Incubation period for egg (in days)
	First	Second	Third	Fourth	Fifth	Male	Female	
P. cattleinum	2.6±0.3	3.0±1.0	3.6±0.6	4.6±0.6	6.3±0.3	16.0±0.7	17.0±0.7	8.6±0.3
C. sinensis	2.0±0.2	1.0±0.1	4.0±0.4	2.0±0.2	5.0±0.5	14.8±0.1	15.8±0.3	6.0±0.6
CD (P = 0.05)	NA	NA	NA	1.901	0.950	NA	NA	0.950

Table 2. Longevity and fecundity of *Helopeltis theivora* on *P. cattleianum* and *C. sinensis*

Host	Longevity (in days)		Fecundity
	Male	Female	(No of eggs laid)
P. cattleianum	22 ± 0.57	26 ± 0.58	90 ± 2.5
C. sinensis	17 ± 0.56	21 ± 0.57	120 ± 2.1
CD (P = 0.05)	2.328	2.328	5.345

young shoots of *P. cattleianum* and *C. sinensis* and in the bottle (25 cm x 11 cm) separately. The shoots with the eggs were kept for hatching. After hatching the nymphs were reared in different glass bottles (11.0 cm x 5.5 cm) closed with nylon mesh to prevent migration. The fresh *P. cattleianum* and *C. sinensis* shoots kept in water filled vial were supplied as food on every alternate day. Observations were made on the morphological changes during incubation period and duration of nymphal instars. The newly emerged males and females from the nymphs were separated and kept for pairing. The matted adult was fed with fresh tender shoots of *P. cattleianum* and *C. sinensis*. The process was repeated twice and observations were made on various biological parameters.

Comparative study of developmental stages of *H. theivora* on *Camellia sinensis* and *P. cattleianum* revealed that the developmental period was lower on *C. sinensis* than *P. cattleianum*. There was no significant variation in the developmental periods of I, II and III instars on both host plants. The study was in line with Roy *et al*. (2009) who recorded the nymphal development period varied from 8.4 to 16.2 days. Even though the developmental periods of male and female were longer on *P. cattleianum* there was no significant difference on both host plants (Table 1). Incubation period was prolonged on *P. cattleianum* (8.6 ± 0.33 days) than *Camellia sinensis* (6.0 ± 0.6 days).

Female showed longer longevity when fed with *P.cattleianum* (26 days) than *C. sinensis* (21 days). And male showed less lifespan when fed with *P. cattleianum* (22 days) than *C. sinensis* (17 days) *Psidium* (Table 2). The significant different between the *P.cattleianum* and *C. sinensis* are same respectively. The result is in accordance with the study of Sudhakaran and Muraleedharan (2006) who recorded the average longevity of females was 48 days whereas males lived for only 28 days in tea. Fecundity was lower on *P. cattleianum* than *C. sinensis*.

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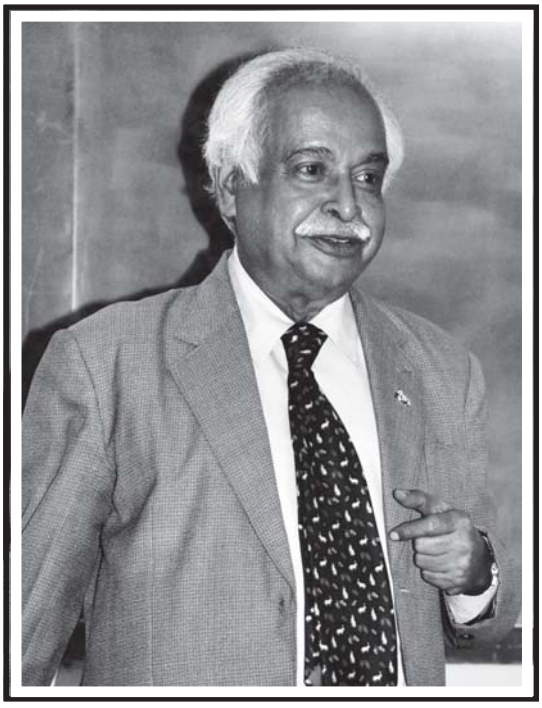

Dr. Ashish Kumar Ghosh
(1938- 2018)

Dr. Asish Kumar Ghosh (b. 1938) former Director, Zoological Survey of India and founder Director, Centre for Environment and Development (ENDEV), Kolkata passed away in Kolkata on April 1, 2018 at the age of 81. A prolific reader and an orator *par excellence*, he became silent for a brief period and was battling throat cancer.

A bachelor, Dr. Ghosh had his early education in Rourkella, Odisha, and later had his graduation (1957), Masters (1959) and Ph. D. (1964) from the University of Calcutta. After teaching in an undergraduate college in Kolkata for a brief period, he moved to the Department of Plant Pathology and Entomology, University of Wisconsin, Madison, USA as a 'Fulbright Scholar and Rockfeller Foundation Grantee' where he continued his research on 'Long range dispersal of aphid-vectors of plant viruses'. After returning to India, Dr. Ghosh again joined the University of Calcutta as a Research Officer in a PL 480 Project and continued his researches extensively on the taxonomy of aphids.

On joining the Zoological Survey of India in 1972, besides taxonomic works, he became more interested in environmental and biodiversity related works and biodiversity

conservation. The first Environmental Monitoring Wing in ZSI (Kolkata and Chennai) was started under Dr. Ghosh's leadership in early 1980's. Between 1992 - 1996, he led delegations to the Ramsar Convention in Japan and acted as a Member of Indian delegation to the Asian Wetland Conference to Malaysia, Indo- Russia Forest Meet to Russia, IUCN General Assembly to Argentina and also other International meet in Kenya, China, France, Mexico and Spain. He also served as a Member of the Biodiversity Authority of India and took active and important role in formulating Indian Biodiversity Act- 2002 and its Rules in 2004. An outspoken Environmental Activist, Dr. Ghosh had the courage to submit an affidavit supporting the public in wetland case while still he was in office.

Under the leadership of Dr. Ghosh, ENDEV was actively engaged in biodiversity exploration and documentation, exploring old and indigenous varieties of crops, fruits etc. at block level. The first People's Biodiversity Register (PBR) in West Bengal on the biodiversity of Kolkata was prepared through his leadership. After the Sundarban was devastated by cyclone Aila in 2009, Dr. Ghosh worked on the field with the affected people and launched several projects. Out of these, the revival of long-forgotten traditional paddy seeds that grow in brackish water became a life saver. This endeavor won the World Bank honour for best innovation among more than 100 contestant countries.

Dr. Ghosh had written extensively on biodiversity conservation, natural resource management, on different dimensions of environment and development along with his basic interest on aphids taxonomy. He has more than 400 research papers to his credit besides about 10 books and monographs. He also authored seven volumes of 'Fauna Volumes' on Indian Aphids published by the Zoological Survey of India. He won several prestigious honours and prizes and became the President of the Aphidological Society of India.

Dr. Ghosh was also Visiting Faculty Member of several premier institutions. He also supervised a dozen of Ph. D. students. He had keen interest in literature, film and other social activities. His death caused profound grief among a large number of students, academics, environmental scientists and scholars in India and abroad.

Prof. Samiran Chakrabarti
UGC Emeritus Fellow and Professor & Head,
Department of Zoology,
University of Kalyani, Kalyani 741235
Email: chakrabarti32b@gmail.com

AUTHOR INDEX

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